Draft

Report on Carcinogens
Background Document for

Steroidal Estrogens

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

Steroidal Estrogens

Carcinogenicity

Steroidal estrogens are *known to be human carcinogens,* based on sufficient evidence from human epidemiology studies showing that use of estrogen replacement therapy in postmenopausal women is associated with a consistent increase in the risk of endometrial cancer and a less consistent increase in the risk of breast cancer. Higher risks of endometrial and breast cancer were associated with longer durations of exposure or higher doses of estrogens. Some evidence suggests that oral contraceptive use also may be associated with increased risk of breast cancer. The evidence in humans for the carcinogenicity of steroidal estrogens is supported by findings from experimental animal studies that have shown a variety of neoplasms including endometrial, cervical, and mammary tumors in mice, mammary and pituitary neoplasms in rats, and renal carcinomas in hamsters.

The carcinogenic effects of hormone replacement therapy used to relieve symptoms of menopause were evaluated by the International Agency for Research on Cancer (IARC) (1999). Most of the studies reviewed did not differentiate between the effects of estrogen-only and estrogen–progestin combination therapies. An increased risk of endometrial cancer was associated with increasing duration of therapy. A small increased risk of breast cancer also was found. One cohort and three large case-control studies not included in the IARC (1999) review reported an association of estrogen replacement therapy with endometrial cancer risk (Persson *et al*. 1999, Cushing *et al*. 1998, Shapiro *et al*. 1998, Weiderpass *et al*. 1999); the latter two studies both reported stronger positive associations between estrogen replacement therapy and endometrial cancer with increasing duration of estrogen use. Three recent cohort studies of the effects of hormone replacement therapy have shown an association with breast cancer (Schairer *et al.* 2000, Persson *et al.* 1999, Gapstur *et al.* 1999). Two of four recent case-control studies found that estrogen-only replacement therapy was associated with increased risk of breast cancer (Magnusson *et al.* 1999, Henrich *et al.* 1998), whereas Brinton *et al*. (1998) reported a slight protective effect of hormone replacement therapy (estrogen content not specified) on breast cancer risk, and Titus-Ernstoff *et al*. (1998) found no association with breast cancer risk. One recent study (Purdie *et al*. 1999) found that estrogen therapy was associated with increased risk for ovarian cancer. In general, the results of recent studies are consistent with previously reviewed studies of estrogen use (IARC 1999).

Numerous case-control and cohort studies have addressed the risks of various cancers associated with the use of oral contraceptives (IARC 1999). Most of these studies involved estrogen–progestin combinations. In general, oral contraceptive use was associated with a small increased risk of breast cancer. Three recent case-control studies (Titus-Ernstoff *et al*. 1998, Brinton *et al*. 1998, Rohan and Miller 1999) did not support the positive association between oral contraceptive use and breast cancer suggested in the earlier studies reviewed by the IARC (1999). However, an inverse association between oral contraceptive use and ovarian and endometrial cancer was recently reported

(Salazar-Martinez *et al.* 1999), confirming the IARC review. None of the recent studies specified the hormone content of the oral contraceptives used.

Studies in rats, mice, hamsters, and guinea pigs have been conducted with estrogens alone or in combination with known carcinogens. Estrogen had a carcinogenic effect in all species and by all routes of administration. Most studies showed induction of benign and malignant neoplasms, as well as preneoplastic lesions, in a variety of target organs, including the breast and female reproductive tract.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Although there is no evidence suggesting genotoxic effects in nonmammalian systems (IARC 1999), steroidal estrogens can damage chromosomes and DNA in mammals. The most frequently reported effects include DNA adduct formation, cytogenic alterations (*e.g.*, chromosome and chromatid breaks, micronuclei, SCEs), aneuploidy, and cell transformation. Most of these effects have been demonstrated in various *in vitro* assays using cultured animal cells or cell-free systems. Fewer effects have been reported in whole-animal studies or in studies with human cells, and no human *in vivo* studies were identified.

Estrogen metabolism is essentially similar among mammalian species, with aromatic hydroxylation to catechol intermediates and glucuronidation, sulfonation, and *O*-methylation.

Although there is strong evidence that estrogen carcinogenesis is mediated by the estrogen receptor, there is evidence that this activity alone is insufficient to explain the carcinogenic effects of estrogens in all tissues. Although the molecular mechanisms responsible for estrogen carcinogenicity are not well understood, the evidence indicates that steroidal estrogen carcinogenesis is complex and may involve proliferative effects and direct and indirect genotoxic effects. The relative importance of each mechanism is likely a function of the specific estrogen, as well as the exposed tissue or cell type and its metabolic state (Yager and Liehr 1996).

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1 Introduction

Conjugated estrogens were listed in the Fourth Annual Report on Carcinogens (RoC) (1985) as *known to be human carcinogens.* A number of individual steroidal estrogens, including estradiol-17β, estrone, ethinylestradiol, and mestranol, also were listed in that RoC as *reasonably anticipated to be human carcinogens*. In 1987, the International Agency for Research on Cancer (IARC) identified steroidal estrogens as *carcinogenic to humans* (Group 1), based on sufficient evidence of carcinogenicity in humans (IARC 1987). The IARC noted that its evaluation applied to the group of chemicals as a whole, and not necessarily to all individual chemicals within the group. Also in 1987, and again in 1999, the IARC identified postmenopausal estrogen therapy as *carcinogenic to humans* (Group 1), based on sufficient evidence of carcinogenicity in humans (IARC 1987, 1999). These IARC listings are based on a consistent, strongly positive association between exposure to a number of steroidal estrogenic substances and increased risks of endometrial and breast cancer in women. Steroidal estrogens (including postmenopausal estrogen therapy and oral contraceptives) were nominated for listing in the RoC by the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) RoC Review Group (RG1), based on the IARC listing of steroidal estrogens as *carcinogenic to humans* (Group 1).

1.1 Chemical identification

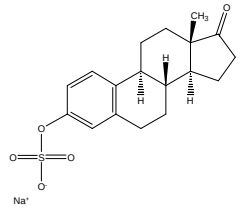
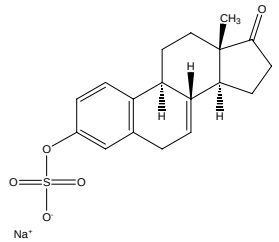
Estrogen is a steroid hormone occurring naturally in both females and males. Hormones are signaling molecules secreted into the bloodstream by endocrine cells; a hormone acts on target cells that possess receptors for that hormone. Steroid hormones are fat-soluble (lipophilic) hormones with a tetracyclic base structure, and are essential for the growth, differentiation, and function of many tissues in both humans and animals. “Estrogen” is a collective term for the female hormones, the most powerful of which is estradiol. These hormones control female secondary sexual characteristics and prepare and maintain the uterine lining. Estrogens affect the growth, differentiation, and function of peripheral tissues of the reproductive system, including the mammary gland, uterus, vagina, and ovary. Estrogens also play an important role in bone maintenance and exert cardioprotective effects. In the brain, estrogens modulate physiological parameters important for regulating procreation, including reproductive behavior, gonadotropin production and release from the pituitary, and mood. Both naturally occurring and synthetic estrogens are widely used medicinal drugs (IARC 1999). Although estrogen is best known for its critical role in influencing female secondary sexual characteristics, reproductive cycle, fertility, and maintenance of pregnancy, less well known are the important actions of estrogen in male tissues, such as the prostate, testis, and epididymis. In addition to their well-known role in female bone formation and maintenance, estrogens are essential for the normal development of bone tissue in males. Modification of the hormonal environment can increase or decrease the spontaneous occurrence or induction of tumors (IARC 1999).

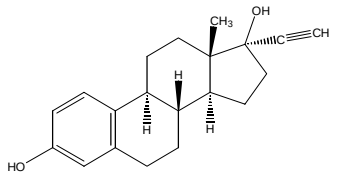
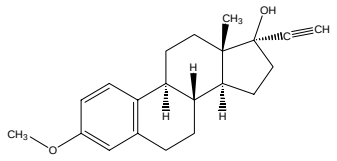
1.2 Physical-chemical properties

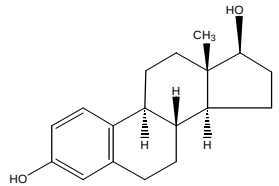
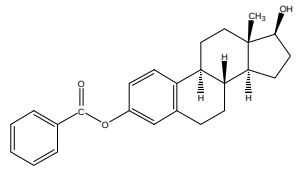
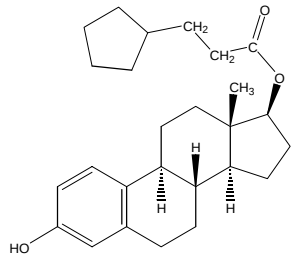
“Conjugated estrogens” (sulfate conjugates) refer to mixtures that contain any of at least eight different compounds, including sodium estrone sulfate and sodium equilin sulfate. These are

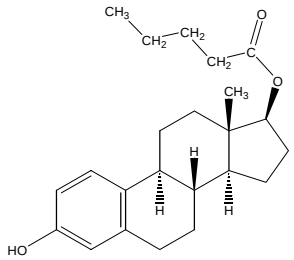
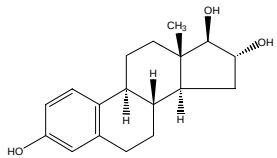
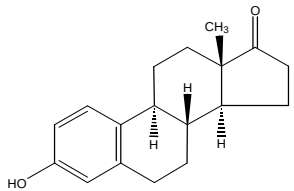
derived wholly or partly from equine urine or synthetically from estrone and equilin. The chemical structures and physical-chemical properties of conjugated estrogens and other commonly used steroidal estrogens are listed in Table 1-1.

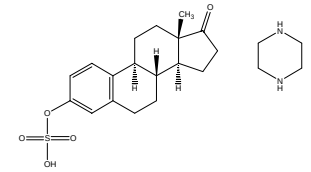
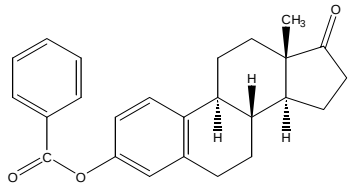
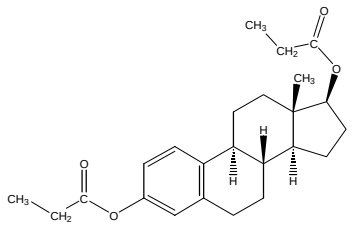
Table 1-1. Physical and chemical properties of estrogens

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Sodium estrone sulfate 438-67-5	3-(sulfooxy)-estra-1,3,5(10)-trien-17-one, sodium salt; estrone sodium sulfate; estrone, hydrogen sulfate sodium salt	$C_{18}H_{21}NaO_5S$ 372.41		buff-colored odorless powder soluble in water
Sodium equilin sulfate 16680-47-0	–	$C_{18}H_{19}NaO_5S$ 370.4		buff-colored odorless powder soluble in water

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Ethinylestradiol 57-63-6	17-ethynyl estradiol; 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol; estone; 19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol, (17α)-; 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17,diol; amenoron; Anovlar; chee-o-genf; 3,17β-dihydroxy-17α-ethynyl-1,3,5(10)-estratriene; diognat-e; diogyn-e; Dyloform; EE; Esteed; Estigyn; Estinyl; estoral (orion); estroals; estra-1,3,5(10)-triene-3,17β-diol, 17α-ethynyl-; Ethidol; ethinoral; 17α-ethynyl-3,17-dihydroxy-δ-(sup1,3,5)-estratriene; Primogyn; Primogyn c (or m); Progynon c; Eticyclin; eticyclol; etinestrol; etinestryl; ginestrene; inestra; Linoral; Lynoral; Menolyn; Neo-Estrone; Novestrol; oradiol; orestralyn; Palonyl; perovex; Feminone; roldiol; Spanestrin; ylestrol; 17α-ethynyl-3-hydroxy-1,3,5(10)-estratrien-17β-ol; ethinylestradiol; ethinyloestradiol	$C_{20}H_{24}O_2$ 296.41		fine white to creamy white odorless crystalline powder melting point, 182–184°C practically insoluble in water (< 0.1 g/100 mL at 21°C) soluble in acetone, ethanol, chloroform, dioxane, diethyl ether, and vegetable oils
Mestranol 72-33-3	ethynylestradiol 3-methyl ether; 3-methoxy-17α-ethynyl-1,3,5(10)-estratriene-17β-ol; 17α ethynyl-estradiol-3-methyl ether; 3-methoxy-19-nor-17α-pregna-1,3,5-trien-20-yn-17-ol; compound 33355; δ-MVE; 3-methylethynylestradiol; 17αl-19-norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-; methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol; norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-	$C_{21}H_{26}O_2$ 310.44		white to creamy white odorless crystalline powder melting point, 150–151°C practically insoluble in water sparingly soluble in ethanol slightly soluble in methanol soluble in acetone, dioxane, and diethyl ether freely soluble in chloroform

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estradiol 50-28-2	β-estradiol; dihydrofolliculin; dihydroxyestrin; 1,3,5(10)-estratriene-3,17β-diol; 3,17-dihydroxy-δ(1,3,5-10)-estratriene; 3,17-epidihydroxyestratriene; estradiol-17β; 17β-estradiol; estra-1,3,5(10)-triene-3,17β-diol	$C_{18}H_{24}O_2$ 272.38		white to creamy white odorless crystalline powder melting point, 173–179°C practically insoluble in water soluble in ethanol, chloroform, diethyl ether, acetone, and dioxane a natural hormone present in pure form in the urine of pregnant mares and in the ovaries of pigs
Estradiol benzoate 50-50-0	estradiol monobenzoate; estradiol benzoate; 17β-estradiol benzoate; estradiol-3-benzoate; 17β-estradiol-3-benzoate	$C_{25}H_{28}O_3$ 376.49		white crystalline powder melting point, 191–196°C practically insoluble in water slightly soluble in ethanol and diethyl ether sparingly soluble in acetone and vegetable oils
Estradiol cypionate 313-06-4	estradiol cyclopentylpropionate; β-estradiol-17-cyclopentanepropionate; 1,3,5(10)-estratriene-3,17β-diol, 17-cyclopentapropionate; Depofemin	$C_{26}H_{36}O_3$ 396.57		white odorless crystalline powder melting point, 151–152°C practically insoluble in water soluble in ethanol, chloroform, diethyl ether, acetone, and dioxane

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estradiol valerate 979-32-8	estradiol-17-valerate; estradiol-17β-valerate	$C_{23}H_{32}O_3$ 396.50		white odorless crystalline powder melting point, 144–145°C practically insoluble in water soluble in benzyl benzoate, dioxane, methanol, and castor oil sparingly soluble in arachis oil and sesame oil
Estriol 50-27-1	drihydroxyestrin; δ(1,3,5-10)-estratriene-3,16-cis-17-trans-diol; 1,3,5(10)-estratriene-3,16α,17β-triol; estra-1,3,5(10)-triene-3,16α,17β-triol; estriol (R&D)	$C_{18}H_{24}O_3$ 288.39		white odorless crystalline powder melting point, 282°C practically insoluble in water sparingly soluble in ethanol soluble in acetone, dioxane, diethyl ether, and vegetable oils
Estrone 53-16-7	folliculin; ketohydroxyestrin; 1,3,5(10)-estratrien-3-ol-17-one; oestrone; β-estrone; estra-1,3,5(10)-trien-17-one, 3-hydroxy-; estrol; oestrin; 3β-hydroxyestra-1,3,5(10)-trien-17-one; 3-hydroxy-1,3,5(10)-estratrien-17-one	$C_{18}H_{22}O_2$ 270.37		white to creamy white crystalline powder melting point, 254.5–256°C practically insoluble in water sparingly soluble in ethanol, chloroform, acetone, dioxane, and vegetable oils slightly soluble in diethy ether and solutions of alkali hydroxides

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estropipate 17280-37-7	piperazine estrone sulfate; 3-(sulfooxy)estra-1,3,5-(10)-trien-17-one compd. with piperazine (1:1); estrone, hydrogensulfate compd. with piperazine (1:1); Harmogen; Ogen; piperazine 17-oxo-estra-1,3,5(10)-trien-3-yl sulfate; sulestrex piperazine	$C_{22}H_{32}N_2O_5S$ 436.56		white to yellowish white odorless fine crystalline powder melting point, 190°C; solidifies on further heating and decomposes at 245°C very slightly soluble in water (0.08 g/100 mL), ethanol, chloroform, and diethyl ether soluble in warm water and warm ethanol
Polyestradiol phosphate 28014-46-2	(17b)-estra-1,3,5(10)-triene-3,17-diol polymer with phosphoric acid	NA	NA	melting point, 195 °C
Estrone benzoate 2393-53-5	3-(benzoyloxy)estra-1,3,5(10)-trien-17-one	$C_{25}H_{26}O_3$ 374.48		melting point, 220 °C
Estradiol dipropionate 113-38-2	alpha-estradiol dipropionate; 17β-estradiol dipropionate; estral,3,5(10)-triene-3,17-diol(17β)-dipropionate	$C_{24}H_{32}O_4$ 384.5144		melting point, 104 °C

Source: IARC 1987 and 1999, ChemFinder 2000

1.3 Identification of metabolites

Administered estrogens and their esters are handled within the body essentially in the same way as the endogenous hormones. Metabolic conversion of estrogens occurs in the liver and at local target tissues (FDA 1999). Although naturally occurring estrogens circulate in the blood largely bound to sex hormone–binding globulin and albumin, only unbound estrogens enter target-tissue cells. Section 6 provides more information on the metabolic pathways.

2 Human Exposure

2.1 Use

Steroidal estrogens comprise a group of structurally related hormones derived from the cholesterol molecule. They control sex and growth characteristics, are highly lipophilic, and elicit biological responses by binding to nuclear receptors that act as DNA transcription factors.

2.1.1 Hormone replacement therapy

Conjugated estrogens, estradiol, and synthetic esters of estradiol, especially estradiol valerate, are most commonly used for estrogen replacement therapy to treat symptoms of menopause, including menopause surgically induced by removal of the ovaries. They are used to prevent the sweating episodes called hot flashes and the shrinking and irritation that sometimes occur in the vulva, vagina, and urinary organs. Estrogens also can be used to prevent common post-menopausal conditions such as osteoporosis and ischemic heart disease. They also have been used to treat hypoestrogenism due to hypogonadism, castration, or primary ovarian failure. Estrogen replacement therapy can employ steroidal estrogens only or a combination of steroidal estrogens and progestogens (IARC 1999, FDA 1999, HSDB 2000). Steroidal estrogens used for hormone replacement therapy (HRT) are summarized in Table 2-1.

Table 2-1. Commonly prescribed estrogens used for hormone replacement therapy in the United States.

Estrogens	Brand	Strength (mg)	Manufacturer
Conjugated estrogens	Premarin	0.3, 0.625, 0.9, 1.25, 2.5	Wyeth-Ayerst
Vaginal cream	Premarin Vaginal Cream	625	Wyeth-Ayerst
Esterified estrogens	Estratab	0.3, 0.625, 1.25, 2.5	Solvay
	Menest	0.3, 0.625, 1.25, 2.5	SmithKline Beecham
17 β-Estradiol			
Transdermal patch	Estraderm	0.05, 0.10	Ciba-Geneva
Transdermal patch	Climara	0.05, 0.10	Berlex
Transdermal patch	Vivelle	0.0375, 0.05, 0.075, 0.10	Ciba-Geneva
Vaginal cream	Estrace	1000	Bristol-Myers Squibb
Estradiol, micronized	Estrace	0.5, 1.2	Bristol-Myers Squibb
Estropipate	Orgen	0.75, 1.5, 3	Upjohn
	Ortho-Est	0.75, 1.5	Ortho
Vaginal cream	Ogen	1000	Upjohn
Combination Therapy			
Conjugated estrogens + MPA, continuous combined regimen[a]	Prempo	0.625/2.5	Wyeth-Ayerst
Conjugated estrogens + MPA, cyclic regimen[b]	Premphase	0.625/5	Wyeth-Ayerst

Source: Klein and Berlin 1996
[a]Conjugated estrogens and medroxyprogesterone acetate (MPA) taken daily.
[b]Conjugated estrogens taken daily, MPA taken for last half of 28-day cycle.

2.1.2 Oral contraceptives

Steroidal estrogens, most commonly ethinylestradiol, also are used with various progestogens in combined oral contraceptive (OC) formulations. Estrogens have been used in oral contraceptives for over 30 years. During the 1960s and 1970s, research was done to attempt to reduce the estrogen content of oral contraceptives, because of the risks of thromboembolic disorders associated with the use of high doses of estrogens. Currently, many of the oral contraceptives used in the United States contain either 30 or 35 μg of ethinylestradiol, because this dose has contraceptive efficacy, good tolerability, and a low risk of adverse effects such as breakthrough bleeding (Schwend and Lippman 1996). Mestranol also is used in some formulations of oral contraceptives (IARC 1999). Combined oral contraceptives usually are administered as a pill taken daily for 20 to 22 days followed by a 7-day pill-free interval, where a withdrawal bleed is expected to occur.

Daily administration of a mixture containing ethinylestradiol for five consecutive days can prevent pregnancy if given within 72 hours after coital exposure (IARC 1999, HSDB 2000). Appendix A (Annex 2, Table 1) summarizes information relating to combinations of estrogens used in oral contraceptives.

2.1.3 Other uses

Steroidal estrogens are used to treat breast cancer (for palliation only) in selected women and men with metastatic disease. They also are used in palliative treatment of androgen-dependent carcinoma of the prostate. Use of estrogens to treat acne is not recommended, because of lack of evidence for efficacy. Veterinarians use estrogens to induce ovulation and estrus in animals. They also can be used to treat anal adenomata and prostatic hypertrophy in male dogs and mesalliance pseudopregnancy, vaginitis, and incontinence in female dogs. Mixed androgen–estrogen therapy is used in canine geriatrics. Steroidal estrogens also are used for biochemical research (Novartis 2000, HSDB 2000).

2.2 Production

Steroidal estrogens are produced from estrogens obtained from the urine of pregnant mares or synthetically. The principal estrogen present in conjugated estrogens is sodium estrone sulfate (between 52.5% and 61.5%). The estrogenic potency of the conjugated estrogens is expressed as the equivalent quantity of sodium estrone sulfate. Conjugated estrogens also contain sodium equilin sulfate (between 22.5% and 30.5%). Ethinylestradiol is formed by treatment of estrone with potassium acetylide in liquid ammonia. Mestranol is prepared by reaction of estrone with methyl sulfate to produce its 3-methoxy analogue (IARC 1999).

2.3 Analysis

Gas chromatography with flame ionization detection is used to identify steroidal estrogens, their components, and impurities. Infrared and ultraviolet absorption spectrophotometry and thin-layer chromatography are the most common methods used to identify ethinylestradiol, mestranol, estradiol, estriol, estrone, and estropipate. Liquid chromatography and high-pressure liquid chromatography usually are used to assay their purity. Thin-layer chromatography, liquid chromatography, ultraviolet absorption spectrophotometry, and potentiometric titration are used to determine purity and content of various steroidal estrogens in pharmaceutical preparations (IARC 1999).

2.4 Environmental occurrence

Steroidal estrogens are naturally occurring hormones that stimulate growth and development of the female sex organs in vertebrates. Under normal conditions, estrogens are synthesized in the ovaries in response to pituitary hormones. In a normally cycling adult woman, the ovarian follicle secretes 70 to 500 μg of estradiol per day, depending on the phase of the menstrual cycle. This estradiol is converted primarily to estrone and small amounts of estriol. After menopause, endogenous estrogen is produced by the conversion of androstenedione secreted by the adrenal cortex to estrone by peripheral tissues (FDA 1999).

Steroidal estrogens and nonsteroidal compounds with estrogenic activity also occur naturally in plants; over 360 plants have been identified as possessing estrogenic activity.

Estrogens have been found naturally in such plants as licorice, French bean, date palm, pomegranate, and apples. A few plants contain the principal mammalian estrogens, estradiol and estrone (Satchell 1985). Screens have been established to determine estrogen content in meat and milk. Estradiol equivalent concentrations in meat and milk were determined by a uterine estrogen receptor assay (a competitive protein binding assay). Meat, including chicken, pork, and beef, was shown to contain 57 ± 29.5 pg of estradiol equivalents (range 38 to 88 pg, n = 144), and milk to contain 53 ± 6.8 pg of estradiol equivalents (range 35 to 65 pg, n = 81) (Collins and Musey 1985). Veterinary use of steroidal estrogens (for growth promotion and therapeutic purposes) can increase tissue levels in food-producing animals above those resulting from endogenous estrogen production.

2.5 Environmental fate

Information about the environmental fate of steroidal estrogens was not identified in the current literature. The biological fate of estrogens is discussed in Section 2.8, below.

2.6 Environmental exposure

Estrogens are responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Although circulating estrogens exist in a dynamic equilibrium of metabolic interconversions, estradiol is the main naturally occurring estrogen. Estradiol is substantially more potent than its metabolites estrone and estriol at the receptor level. The primary source of estrogen in a normally cycling adult woman is the ovarian follicle, which secretes 70 to 500 μg of estradiol per day, depending upon the phase of the menstrual cycle. After menopause, most endogenous estrogen is produced in the peripheral tissues by the conversion of androstenedione to estrone. Androstenedione is secreted by the adrenal cortex. Thus, estrone, and its sulfate conjugated form, estrone sulfate, are the most abundant circulating estrogens in post-menopausal women (IARC 1999).

Exposure to estrogens in the United States occurs mostly when they are administered in oral contraceptives and to a lesser degree in post-menopausal estrogen therapy. In the United States, 15% of women in 1990 used oral contraceptives containing estrogens. Of the 35,800,000 women in the United States in 1990, about 5,191,000 used oral contraceptives. The use of post-menopausal estrogen therapy became widespread in the United States in the 1960s. Between 1962 and 1967, the number of women using this therapy increased by 240%. By 1967, approximately 13% of the women in the United States aged 45 to 64 used this type of therapy. Estrogen–androgen combinations accounted for an estimated 14% of noncontraceptive prescriptions in the United States in 1966, but by 1983, the percentage had fallen to < 2%. The number of estrogen-androgen prescriptions then began to rise again, from 0.1 million in 1982 to 0.8 million in 1992 (IARC 1999).

2.7 Occupational exposure

No information about occupational exposure to estrogens was found in the current literature.

2.8 Biological indices of exposure

Estrogens, like all steroid hormones, have a wide range of actions and affect almost all systems in the body, yet act in a tissue-specific manner. Although estrogen's mode of action has been studied extensively, the molecular mechanism of action still is unclear. Estrogen acts by binding with high affinity and high specificity to the protein receptors present in hormone-responsive tissues. When the hormone binds with the receptor, the receptor undergoes a conformational change and binds to specific DNA sequences. This transcription complex regulates the expression of specific genes within a cell (Edwards and Prendergast 1996). Circulating estradiol and other naturally occurring estrogens are bound mainly to the sex hormone binding globulin, and to a lesser degree to albumin (Novartis 2000).

Estrogens, whether exogenous or endogenous, circulate in the body, undergoing various metabolic interconversions. Estrogens undergo enterohepatic recirculation via sulfate and glucuronide conjugation in the liver (where most of the transformations take place), biliary secretion of conjugates into the intestine, and hydrolysis in the gut, followed by reabsorption. Estradiol, the most abundant endogenous estrogen, can be converted reversibly to estrone, and both can be converted to the major urinary metabolite, estriol. Estradiol, estrone, and estriol are excreted in the urine, along with glucuronide and sulfate conjugates (Mosby 2000).

When given orally, naturally occurring estrogens and their esters are extensively metabolized by the liver and circulate primarily as estrone sulfate, which limits the potency of orally administered estrogen. Synthetic estrogens, like ethinylestradiol, are degraded very slowly in the liver and other tissues, resulting in higher innate potency (Mosby 2000). Estradiol has a peak plasma level at 2 to 4 hours after administration, with a plasma half-life of 24 hours (Infomed-Verlags AG 1996).

2.9 Regulations

The U.S. Food and Drug Administration (FDA), through the Federal Food, Drug, and Cosmetic Act, regulates manufacturers, packers, and distributors to ensure proper labeling, certification, and usage requirements for any drug containing steroidal estrogens. The FDA also describes specifications and conditions of use for injectable or implantable formulations containing steroidal estrogens for animals, and sets estradiol tolerances in tissues of heifers, steers, calves, and lambs. FDA regulations are summarized in Table 2-2.

Table 2-2. FDA regulations

Regulatory action	Effect of regulation and other comments
21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	The regulations govern the proper labeling procedures for a drug and drug product. For drugs containing estrogen and its derivatives, no new drugs may be released for interstate commerce without proper labeling.
21 CFR 201.301—Notice to manufacturers, packers, and distributors of estrogenic hormone preparations. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	Some drug preparations fabricated wholly or in part from estradiol and labeled as to potency in terms of international units or in terms of international units of estrone activity have been marketed. The declaration of the estradiol content of an estrogenic hormone preparation in terms of weight is considered appropriate.
21 CFR 201.313—Estradiol labeling. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	"Estradiol" and that which is said to be "17-cis-beta estradiol" is the same substance formerly recognized in the United States Pharmacopeia under the designation "Alpha Estradiol." The substance should no longer be referred to in drug labeling as "Alpha Estradiol."
21 CFR 310—PART 310—NEW DRUGS. Promulgated: 39 FR 11680, 03/29/74. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e, 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	Regulations govern the administrative rulings and decisions on new drug status, new drugs exempted from prescription-dispensing requirements, records, reports, and requests for specific new drugs or devices.
21 CFR 310.515—Patient package inserts for estrogens. Promulgated: 55 FR 18723, 05/04/90. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e, 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	The FDA concludes that the safe and effective use of drug products containing estrogens requires that patients be fully informed of the benefits and risks involved in the use of these drugs. Accordingly, each estrogen drug product restricted to prescription distribution, including products containing estrogens in fixed combinations with other drugs, shall be dispensed to patients with a patient package insert containing information concerning the drug's benefits and risks. An estrogen drug product that does not comply with the requirements of this section is misbranded under section 502(a) of the Federal Food, Drug, and Cosmetic Act.
21 CFR 522—PART 522—IMPLANTATION OR INJECTABLE DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 40 FR 13858 03/27/75. U.S. Codes: 21 U.S.C. 360b.	This part regulates specifications, indications, and conditions of use and limitations of animal drugs. The subpart affects estrogen injections.

Regulatory action	Effect of regulation and other comments
21 CFR 522.840—Estradiol. Promulgated: 57 FR 41861, 08/14/92. U.S. Codes: 21 U.S.C. 360b.	Estradiol is used for implantation in steers and heifers as follows: For increased rate of weight gain in suckling and pastured growing steers; for improved feed efficiency and increased rate of weight gain in confined steers and heifers. Each silicone rubber implant contains 25.7 or 43.9 mg of estradiol. Limitations: For subcutaneous ear implantation in steers and heifers only. A second implant may be used if desired. No additional effectiveness may be expected from reimplanting in less than 200 days for the 25.7-mg implant or 400 days for the 43.9-mg implant. Increased sexual activity (bulling, riding, and excitability) has been reported in implanted animals.
21 CFR 522.842—Estradiol benzoate and testosterone propionate in combination. Promulgated: 61 FR 5506, 02/13/96. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in steers and heifers for growth promotion and improved feed efficiency. Dosage includes 20 mg of estradiol benzoate. Limitations: For heifers weighing 400 lb or more; for subcutaneous ear implantation, one dose per animal; not for use in dairy or beef replacement heifers.
21 CFR 522.850—Estradiol valerate and norgestomet in combination. Promulgated: 54 FR 1165, 01/12/89. U.S. Codes: 21 U.S.C. 360b.	This combination is used for synchronization of estrus and ovulation in cycling beef cattle and non-lactating dairy heifers. An injectable solution (sesame oil) contains 3.0 mg of norgestomet and 5.0 mg of estradiol valerate per 2 mL. Limitations: Insert implant subcutaneously in the ear only; then immediately inject solution intramuscularly only. Counting the day of implantation as day 1, remove the implant on day 10. Collect all implants as they are removed and burn them. While animals are restrained for artificial insemination, avoid other treatments such as vaccinations, dipping, pour-on grub and louse prevention, spraying, etc. For insemination without estrus detection, the entire treated group should be started at 48 hours after the last implant has been removed and should be completed within 6 hours. Where estrus detection is preferred, insemination should be approximately 12 hours after first detection of estrus. Those that do not conceive can be re-bred when they return to estrus approximately 17 to 25 days after implant removal. Do not use in cows producing milk for human consumption.
21 CFR 522.1940—Progesterone and estradiol benzoate in combination. Promulgated: 62 FR 8372, 02/25/97. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in animals to increase rate of weight gain. Amounts used are 100 mg of progesterone and 10 mg of estradiol benzoate per dose. Limitations: For animals weighing 400 lb or more; for subcutaneous ear implantation, one dose per animal, and for additional improvement in rate of weight gain in steers fed in confinement for slaughter, reimplant at approximately day 70.

Regulatory action	Effect of regulation and other comments
21 CFR 522.2477—Trenbolone acetate and estradiol. Promulgated: 62 FR 28629, 05/27/97. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in animals to increase rate of weight gain and improve feed efficiency in feedlot steers. Amounts used include 120 mg of trenbolone acetate and 24 mg of estradiol (6 pellets, each pellet containing 20 mg of trenbolone acetate and 4 mg of estradiol) per animal. Limitations: Implant subcutaneously in ear only. Not for use in animals intended for subsequent breeding or in dairy animals.
21 CFR 522.2478—Trenbolone acetate and estradiol benzoate. Promulgated: 61 FR 29479, 06/11/96. U.S. Codes: 21 U.S.C. 360b.	This combination is used in implantation in animals for improved feed efficiency in steers fed in confinement for slaughter. Amounts used are 200 mg of trenbolone acetate and 28 mg of estradiol benzoate (one implant consisting of 8 pellets, each pellet containing 25 mg of trenbolone acetate and 3.5 mg of estradiol benzoate) per animal. Limitation: Implant subcutaneously in ear only.
21 CFR 556—PART 556--TOLERANCES FOR RESIDUES OF NEW ANIMAL DRUGS IN FOOD. Promulgated: 40 FR 13942 03/27/75. U.S. Codes: 21 U.S.C. 342, 360b, 371.	Tolerances are established based upon residues of the new drugs in the treated edible products of food-producing animals. All of these drugs have been shown to directly or indirectly (through metabolites) induce cancer when ingested by humans or animals.
21 CFR 556.240—Estradiol and related esters. Promulgated: 56 FR 67175, 12/30/91. U.S. Codes: 21 U.S.C. 342, 360b, 371.	No residues of estradiol, resulting from the use of estradiol or any of the related esters, are permitted in excess of the following increments above the concentrations of estradiol naturally present in untreated animals: (a) In uncooked edible tissues of heifers, steers, and calves: (1) 120 parts per trillion for muscle, (2) 480 parts per trillion for fat, (3) 360 parts per trillion for kidney, (4) 240 parts per trillion for liver. (b) In uncooked edible tissues of lambs: (1) 120 parts per trillion for muscle, (2) 600 parts per trillion for fat, kidney, and liver.

Source: The regulations in this table have been updated through the 1999 Code of Federal Regulations 21 CFR, 1 April 1999.

3 Human Cancer Studies

3.1 IARC evaluation

In 1999, the IARC critically reviewed numerous case-control and cohort studies that evaluated the relationship of oral contraceptives and hormone replacement therapy (HRT) to the risk of various cancers (see Appendix A). Breast, cervical and endometrial cancers were the most commonly evaluated cancers in relation to exogenous estrogen use. Most studies of oral contraceptive use or HRT, have, however, been unable to evaluate estrogen use specifically and have instead been limited to investigations of various estrogen-progestin combinations (IARC 1999).

The IARC (1999) concluded that the use of oral contraceptives was associated with a very small increased risk of breast cancer, independent of other gynecological risk factors. However, 10 or more years after cessation of oral contraceptive use, breast cancer risk appeared similar to that for women who had never used oral contraceptives. Oral contraceptive users also were shown to be at greater risk of cervical cancer; however, risk estimates were not adequately adjusted for other health and lifestyle factors in these studies.

Despite the increased risk reported for some cancers, hormone use may be protective for others. IARC (1999) indicated that oral contraceptive use nearly halved the risk of endometrial and ovarian cancers. For both cancers, the protective effect of oral contraceptives was greater for longer duration of use and persisted for at least 10 years after cessation of use.

Studies generally have reported no association between oral contraceptives and colorectal cancer, malignant melanoma, or thyroid cancer, although most of these studies have had few exposed cases and limited exposure information overall. Studies of liver cancer have produced mixed results: two studies reported a strong dose-response relationship between oral contraceptive use and benign hepatocellular tumors, while three others showed no association. These studies have generally evaluated the effects of estrogen-progestin combinations, lacking sufficient information to formally evaluate the effects of estrogen alone (IARC 1999).

The IARC (1999) also summarized studies that evaluated cancer risk associated with use of HRT to relieve symptoms of menopause. Some recent studies have evaluated separately the effects of estrogen-only and estrogen-progestin combination therapies; however, others have not had adequate information to do so. Thus, much of what is known from these studies applies to HRT generally, not estrogen therapy specifically.

The studies reviewed by the IARC (1999) generally reported a small increased risk of breast cancer associated with HRT, especially when used recently and for longer than five years. The studies that separately evaluated estrogen-only therapy and estrogen-progestin combinations reported similar risks associated with either type of therapy. However, the dose and type of hormones administered varied considerably and these factors have not been thoroughly evaluated.

Studies consistently reported an increased risk of endometrial cancer associated with increasing duration of estrogen therapy, which remained high 10 years after cessation of therapy. In contrast, studies of cervical, liver, and thyroid cancers, and malignant melanoma showed no association with estrogen replacement therapy. Studies of ovarian and colorectal cancer have produced mixed results. Most studies have shown no relation between estrogen therapy and either ovarian or colorectal cancer; however a few reports have associated estrogen replacement therapy with an increased risk of ovarian cancer and a slightly reduced risk of colorectal cancer.

Although the IARC's review was released only one year ago, several studies have since been published. For the most part, the newer studies, summarized in Tables 3-1 and 3-2, support the IARC's conclusions based on the studies it reviewed (summarized in Appendix A). Although many studies have been published on the relationship between hormone uses and various cancers, this review focuses on studies that were able to evaluate the effects of estrogens specifically and discusses the results for combination therapies only for studies in which estrogen-specific information was not available.

3.2 Hormone replacement therapy

The effects of HRT have been evaluated in several studies of cancer. Hormones used for replacement therapy can be estradiol, conjugated estrogens, other estrogen-only formulas, or combinations of estrogen and progestin. This review focuses primarily on studies of the effects of estrogen-only therapy, which are summarized in Table 3-1.

3.2.1 Breast cancer

Three cohort studies, one in Sweden and two in the United States, have evaluated the relationship between HRT and the risk of breast cancer. All of these studies adjusted for typical reproductive factors related to breast cancer. Schairer *et al.* (2000) identified 2,082 cases of breast cancer among 46,355 post-menopausal women followed in the Breast Cancer Detection Demonstration Project between 1979 and 1989. In general, the use of estrogen replacement therapy was not associated with breast cancer. However, among thinner women (body mass index [BMI] ± 24.4 kg/m^2), breast cancer risk increased moderately with the duration of estrogen use (see Section 6). Thinner women may be more susceptible to the effects of exogenous estrogen, because their endogenous estrogen levels are lower. The duration of estrogen use was not associated with specific tumor histology in this study.

Persson *et al.* (1999) evaluated the risk of breast cancer among 11,231 women prescribed HRT by comparing those who complied with the prescription with those who did not. Breast cancer risk (reported as relative risk, RR) was elevated among women who used estrogen-progestin combinations for more than six years (RR = 1.7, 95% CI = 1.1 to 2.6, n = 44) but not among women who used estrogen alone (RR = 1.1, 95% CI = 0.7-1.7, n = 35). The authors cautioned that women complying with HRT also may be more likely to be screened for breast cancer, potentially resulting in bias in breast cancer detection.

Gapstur *et al.* (1999) identified 1,520 cases of breast cancer among 37,105 post-menopausal women followed in Iowa. This study evaluated the relationship between HRT (estrogen content not specified) and breast cancers of differing prognostic histologies.

HRT was associated with increased risk of breast cancer of favorable histology, but not with risk of ductal *in situ* carcinoma or invasive ductal or lobular carcinoma. This study also evaluated the timing of exposure, but with little power to detect differences. Information on estrogen-only therapy was not available.

Four case-control studies also evaluated the risk of breast cancer associated with HRT. Brinton *et al.* (1998) reported a slight protective effect of HRT (estrogen content not specified) on breast cancer (reported as an odds ratio, OR) in women over 55 years of age (OR = 0.7, 95% CI = 0.5 to 0.9). This study also evaluated the combined effects from HRT and oral contraceptives. A three-fold increased risk of breast cancer among women with who had used oral contraceptives for more than three years and HRT for more than 10 years was observed. A large questionnaire-based case-control study in which the estrogen content of HRT was not specified was reported by Titus-Ernstoff *et al.* (1998). In this study, use of HRT was not associated with an increased risk of breast cancer, regardless of duration of use (< 3 years or >3 years). Both Magnusson *et al.* (1999) and Henrich *et al.* (1998) reported associations between estrogen-only replacement therapy and breast cancer. In a large Swedish case-control study, Magnusson *et al.* (1999) reported that the risk of breast cancer associated with estrogen replacement therapy increased with duration of estrogen use from OR = 1.7 for use < 2 years to OR = 2.7 for use > 10 years. In a smaller Connecticut study of post-menopausal women, Henrich *et al.* (1998) reported that the risk of breast cancer was twice as high among estrogen users than among controls (OR 2.2, 95% CI = 1.2 to 4.2). The effect estimates were slightly higher for non-conjugated than conjugated estrogens and slightly lower for breast cancer *in situ* than for invasive breast cancer. Although this study did not have information on other reproductive factors typically associated with breast cancer, the authors indicated that adjusting for these factors in other studies has not typically altered estimates of risk associated with estrogen use.

3.2.2 Endometrial cancer

Persson *et al.* (1999) evaluated risk of endometrial cancer in the cohort of Swedish women described above. They found a large elevation in risk in women using estrogen only HRT (RR = 4.2, 95% CI = 2.1-8.4, n=27) and a smaller elevation among those using estrogen-progestin combinations (RR = 1.4, 95% CI = 0.6-3.3, n = 11).

All three of the large case-control studies that evaluated the association between estrogen replacement therapy and endometrial cancer supported the positive associations found by the studies reviewed by the IARC (see Appendix A).

Cushing *et al.* (1998) interviewed 484 women with endometrial cancer from the Washington State cancer registry and 780 controls identified through random-digit telephone dialing about their estrogen use. Endometrial cancer was associated with use of conjugated estrogen (OR 5.4, 95% CI = 2.3 to 13.0), primarily among women who had used estrogen therapy within the previous two years. Slightly stronger associations were seen with estrogen doses higher than 1.25 mg. A sensitivity analysis indicated that even with 20% exposure misclassification, the risk of endometrial cancer among women who had had estrogen replacement therapy would be four times that of controls.

Two other large case-control studies, one from Washington State (Shapiro *et al.* 1998) and one from Sweden (Weiderpass *et al.* 1999), collected specific information about estrogen use through questionnaires. Shapiro *et al.* (1998) found the magnitude of the effect of estrogen therapy to be inversely related to tumor grade (OR = 7.8, 5.8, 2.9 for tumor grades I, II, III, respectively). Weiderpass *et al.* (1999) reported that endometrial cancer was strongly associated with use of conjugated estrogens (OR 4.0, 95% CI = 2.5 to 6.4) and estradiol (OR = 2.5, 95% CI = 1.7 to 3.6). Both studies reported stronger positive associations between estrogen replacement therapy and endometrial cancer with increased duration of estrogen use.

3.2.3 Other cancers

Only one recent study has evaluated the association between estrogen replacement therapy and ovarian cancer. Purdie *et al.* (1999) conducted a large interview study in Australia with 793 women who had ovarian cancer and 855 population-based controls. Although estrogen therapy was only modestly associated with all ovarian cancers, the risk of clear-cell epithelial ovarian tumors evaluated separately was significantly increased among estrogen users (OR = 2.6, 95%CI = 1.3 to 4.9). No trend for duration or recency of estrogen use was apparent.

Two recent studies of colon cancer have been conducted among members of California health maintenance organizations (HMOs). Paganini-Hill (1999) surveyed 249 women with and 7,452 women without colorectal cancer about their use of estrogen replacement therapy, and reported only slight inverse associations between estrogen use and colorectal cancer, adjusted for age. Jacobs *et al.* (1999) used pharmacy records to indicate use of estrogen therapy by 341 women with colon cancer and 1,679 controls. No association was found between estrogen therapy and colon cancer. These studies, like earlier ones (Appendix A)**,** do not provide strong evidence for any association between estrogen use and colon cancer.

3.3 Oral contraceptives

In general, the hormone content of oral contraceptives in cancer studies has not been known; however, the contraceptives most likely contained combinations of estrogen and progesterone. Three recent case-control studies in the United States evaluated the association between breast cancer and oral contraceptive use (summarized in Table 3-2).

Titus-Ernstoff *et al.* (1998) and Brinton *et al.* (1998) identified cases of breast cancer through regional cancer registries. Oral contraceptive use was compared between women with breast cancer and population-based controls. Effect estimates were adjusted for reproductive factors typically associated with breast cancer (e.g., age at menarche, parity, age). Titus-Ernstoff *et al.* (1998) evaluated both pre- and post-menopausal breast cancer and Brinton *et al.* (1998) evaluated dose, timing, and duration of use. Although both studies had reasonable power, neither reported marked associations between oral contraceptive use and breast cancer.

Using the National Breast Cancer Screening Cohort in Washington State, Rohan and Miller (1999) evaluated the effect of oral contraceptive use among 1,425 women with benign breast disease, 691 women with benign proliferative epithelial dysplasia, and 5,443

women without either disease. Oral contraceptive use generally was not associated with either proliferative or non-proliferative forms of breast disease. However, contraceptive use for more than seven years was associated with a slightly decreased risk of proliferative forms of breast disease (OR = 0.7, 95% CI = 0.5 to 0.9). A slight increase in the risk of breast disease with atypia also was associated with oral contraceptive use, but was based on a small number of cases.

In general the results of these three studies do not support the positive association between oral contraceptive use and breast cancer suggested in the earlier studies reviewed by the IARC (Appendix A).

Oral contraceptive use was evaluated in a small case-control study of ovarian and endometrial cancer in Mexico (Salazar-Martinez *et al.* 1999). As in previous studies, an inverse association was shown for both types of cancer, especially when oral contraceptives were used for longer than one year.

3.4 Summary

The results of these studies are generally consistent with previous studies of estrogen use (Appendix A). Although early studies were not always able to distinguish between the use of estrogen-only contraceptives or HRT from the use of estrogen-progestin combinations, recent studies are beginning to make this distinction while also considering how dose, duration, and the specific form of estrogen may affect the associated cancer risk. Results from studies of HRT are somewhat more consistent that those from studies of oral contraceptives. The weight of evidence suggests that estrogen use, as HRT by post-menopausal women, is associated with a slight increase in the risk of breast cancer and a stronger increase in the risk of endometrial cancer. Positive and negative associations between estrogens and various other cancers found in previous studies are less consistent.

Table 3-1. Studies of estrogen replacement therapy and cancer of the breast, endometrium, ovaries, and colon

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Schairer *et al*. 2000	breast US cohort 1979—1989	postmenopausal women Breast Cancer Detection Demonstration Project, screening sites throughout US. 2,082 cases 44,273 non-cases	estrogen questionnaire and interview	estrogen only, ever BMI ≤ 24.4kg.m^2 use < 8 yr use 8 < 16 yr use ≥ 16 yr	805 80 82 72	1.1 (1.0—1.3) 1.0 (0.8—1.3) 1.5 (1.2—2.0) 1.6 (1.2—2.2)	Adj. for typical reproductive factors. No association in women with BMI > 24.4 kg/m^2. Duration not associated with extent of invasive disease or tumor histology.
Persson *et al*. 1999	breast and endometrial Sweden cohort 1987—1993	11,231 women prescribed HRT followed using national cancer registry 198 incident breast cancer cases, 66 incident endometrial cancer cases non-compliers and users for < 1 year used as reference group.	estrogen questionnaire	estrogen only, ever breast cancer use 1—6 yr use 6+ yr endometrial cancer use 1—6 yr use 6+ yr estrogen-progestin combination breast cancer use 1—6 yr use 6+ yr endometrial cancer use 1—6 yr use 6+ yr	 23 35 5 27 28 44 6 11	 1.0 (0.6—1.7) 1.1 (0.7—1.7) 0.9 (0.3—2.5) 4.2 (2.1—8.4) 1.4 (0.9—2.3) 1.7 (1.1—2.6) 1.1 (0.4—3.1)	Adj. for age, follow-up time, age at first full-term pregnancy, body mass index, education menopausal age/status. No effect of duration on breast cancer risk. Increased risks associated with combined HRT.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Gapstur *et al.*1999	breast Iowa cohort 1986—1996	women aged 55—69 1,520 cases 35,585 non-cases	HRT, unspec. questionnaire	favorable histol. HRT ≤ 5yr HRT > 5yr past use ≤ 5yr past use > 5yr current use ≤ 5 yr current use > 5 yr	 28 15 - - - -	 1.7 (1.0—2.7) 2.2 (1.2—4.0) 1.4 (0.8—2.6) 2.7 (1.1—6.7) 4.4 (2.0—9.8) 2.6 (1.2—5.9)	Adj. for age, BMI, and other reproductive factors. No relation between HRT and DCIS or invasive cancer, only cancer with favorable histology. Type of hormone not specified.
Magnusson *et al*. 1999	breast Sweden case-control 1993—1995	women aged 50-74 3,345 cases, hospital registries 3,454 controls national registry	estrogen questionnaire and interview	estrogen only, ever use 1—24 mo use 25—60 mo use 61—-120 mo use 120+ mo	150/106 55/42 27/25 22/13 33/18	1.9 (1.5—2.6) 1.7 (1.1—2.6) 1.5 (0.9—2.6) 2.2 (1.1—4.5) 2.7 (1.5—5.0)	Adj. for typical reproductive factors. ORs for estrogen-progestin combinations similar.
Henrich *et al*. 1998	breast Connecticut case-control 1987—1992	postmenopausal women aged 45+ 109 cases of *in situ* or invasive cancer 545 controls screening from regional sites	estrogen questionnaire	invasive cancer estrogen only, ever conjugated nonconjugated	 19/51 12/44 9/23	 2.2 (1.2—4.2) 1.9 (0.9—4.1) 2.5 (1.0—5.9)	Not adjusted for typical reproductive factors. ORs slightly lower when *in situ* cases included.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Brinton *et al*. 1998	breast Atlanta, GA case-control 1990—1992	women aged < 55 1,031 cases, registry 919 controls random-digit dialing	estrogen and HRT, unspec. interview	estrogen only, ever HRT, unspec. + oral contraceptive use HRT, unspec. > 10 yr + oral contraceptive use > 3 yr	98/122 179/178 25/?	0.7 (0.5—0.9) 1.0 (0.7—1.4) 3.2 (1.4—7.4)	Evaluation of joint effects of OC and HRT indicated positive association when both used for a longer time, but no independent effects. Adj. for typical reproductive factors.
Titus-Ernstoff *et al*. 1998	breast Northeast, U.S. case-control 1988—1991	women aged 20—72 1,636 premenopausal and 4,992 postmenopausal cases, population-based registries 2,760 premenopausal and 6,391 postmenopausal controls driver's license and Medicare lists	HRT, unspec. questionnaire	postmenopausal cancer use ≤ 3yr use > 3yr	 15/14 15/16	 1.1 (0.9—1.2) 0.9 (0.8—1.1)	Adj. for typical reproductive factors. Type of hormones not specified.
Shapiro *et al*.1998	endometrial Washington State. case-control 1985—1991	women aged 45—74 730 cases, state registry 1,002 controls random-digit dialing	estrogen questionnaire	estrogen only use < 3yr use ≥ 3yr tumor grade I tumor grade II tumor grade III	 21/85 93/96 115/96 104/96 28/96	 1.9 (1.1—3.3) 8.4 (5.7—12.4) 7.8 (5.4—11.4) 5.8 (4.0—8.3) 2.9 (1.7—4.8)	Adj. for age and BMI

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Cushing *et al.*1998	endometrial Washington State case-control 1985—1996	Women agee 45—54 484 cases, state registry 780 controls random-digit dialing	estrogen interview	conjugated estrogen dose 0.625 mg ≤ 2 yr since use > 2 yr since use dose 1.25 mg ≤ 2 yr since use > 2 yr since use	18/8 57/24 14/19 34/7 24/37	5.4 (2.3—13.0) 6.0 (3.6—10) 1.6 (0.8—3.3) 12.6 (5.4—29.2) 1.5 (0.8—2.6)	Adj. for typical reproductive factors. Recent users at any dose at higher risk than those > 2 yr since use. Unspec. HRT decreased risk, but unopposed estrogen increased risk.
Weiderpass *et al*. 1999	endometrial Sweden case-control 1994—1995	women aged 50—74. 789 cases, registry 3,368 controls, population	estrogen questionnaire	estrogen only, ever use 2—4 yr use 5—9 yr use 10—14 yr use 15+ yr conjugated estrogen estradiol	98/177 16/41 16/23 15/12 23/11 46/51 55/125	3.2 (2.4—4.4) 2.1 (1.1—4.0) 3.3 (1.6—6.6) 8.4 (3.7—19.2) 12.6 (5.8—27.2) 4.0 (2.5—6.4) 2.5 (1.7—3.6)	Increased effects with increasing dose and duration, but not recency of use. Effects slightly stronger for high doses, but trends for duration similar.
Purdie *et al*. 1999	ovarian Australia case-control 1990—1993	women aged 18—79 793 cases, clinic registry. 855 controls, population	estrogen interview	all ovarian cancer epithelial clear cell	68/662 18/132	1.3 (0.9—1.9) 2.6 (1.3—4.9)	Adj. for typical reproductive factors. No duration or recency trend.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Paganini-Hill	colorectal	women aged 44—98	estrogen	estrogen only, ever	129	0.8 (0.6—1.0)	Adj. only for age.
1999	California	249 cases		dose ≤ 0.625 mg	29		
	cohort	7,452 non-cases	questionnaire	dose ≥ 1.25 mg	42	0.6 (0.4—0.9)	
	1981—1985			last use ≤ 15 yr	51	0.8 (0.5—1.1)	
				2—14 yr	43	1.0 (0.8—1.4)	
				0—1 yr	32	0.7 (0.5—1.0)	
						0.7 (0.4—1.0)	
Jacobs *et al*.1999	colon	women aged 55—79 through HMO	estrogen	estrogen			Adj. only for age.
	California	341 cases		1—749 tablets	21/17	0.9 (0.5—1.4)	
	case-control	1,679 controls	pharmacy records	≥ 750 tablets	28/129	1.1 (0.7—1.7)	
	1984—1993			conjugated estrogen			
				< 375 mg	18/112	0.8 (0.5—1.3)	
				> 375 mg	30/112	1.3 (0.7—2.0)	

Table 3-2. Studies of oral contraceptive use and cancer of the breast, endometrium, or colon

Reference	Cancer type study design and period	Study Population	Exposure information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95%CI)	Comments
Titus-Ernstoff *et al*. 1998	breast Northeast, U.S. case-control 1988—1991	women aged 20—72 1,636 premenopausal cases and 4,992 postmenopausal cases population-based registries. 2,760 premenopausal and 6,391 post-menopausal controls drivers license and Medicare lists.	oral contraceptive, unspecified interview	premenopausal use < 3 yr use > 3 yr postmenopausal use ≤3 yr use > 3 yr	 44/45 32/30 11/12 7/7	 (0.9—1.3) (0.9—1.2) (0.9—1.2) (0.9—1.2)	Adj. for typical reproductive factors. Type of hormones not specified.
Brinton *et al*. 1998	breast Atlanta, GA case-control 1990—1992	women aged < 55 1,031 cases, registry. 919 random digit dialing controls	oral contraceptive, unspecified interview	Oral contraceptive ever use 5—9 yr use 10+ yr first use < 15 yr first use 15-19 yr first use 20+ yr	748/641 231/204 173/127 71/56 165/125 512/460	1.1 (0.9—1.4) 1.1 (0.9-1.4) 1.3 (0.9—1.7) 1.3 (0.8—2.1) 1.3 (0.9—1.8) 1.1 (0.9—1.4)	Hormones in oral contraceptive not specified. Adjusted for typical reproductive factors.
Rohan and Miller 1999	breast Washington case-cohort	women aged 40—49 National Breast Cancer Screening Study Cohort 1,425 benign breast disease cases, 691 benign proliferative epithelial disorder cases, 5,443 non-cases.	oral contraceptive, unspecified questionnaire	nonproliferative proliferative without Atypia with Atypia	877/548 424/267 229/359 19/50	1.0 (0.9—1.1) 0.9 (0.8—1.1) (0.8—1.1) 1.5 (0.9—2.7)	Inverse association for proliferative forms of benign breast disease, increased with duration of use. No relation between duration and benign proliferative epithelial disorder

Reference	Cancer type study design and period	Study Population	Exposure information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95%CI)	Comments
Salazar-Martinez *et al*. 1999	endometrial and ovarian Mexico case-control 1995—1997	women attending hospital clinic. 84 ovarian cancer, 85 endometrial cancer 668 clinic/age-matched controls	oral contraceptive, unspecified questionnaire	ovarian cancer use 1-12 mo use ≥13 mo endometrial cancer use 1-12 mo use ≥13 mo	 6/78 7/117 6/78 7/117	 0.6 (0.2—1.3) 0.4 (0.2—0.8) 0.5 (0.2—1.4) 0.4 (0.1—0.9)	Adj. for typical reproductive factors. Type of hormones not specified.

4 Studies of Cancer in Experimental Animals

The International Agency for Research on Cancer (IARC) reviewed carcinogenicity studies of estrogens (conjugated estrogens, estradiol, estriol, estrone, and synthetic estrogens) in experimental animals. These substances were tested via oral administration (diet and drinking water), subcutaneous injection, and implantation (IARC 1999, 1987, 1979; Appendices A, B and C). A summary of the results of these studies is presented in Table 4-1. An overview of the studies reviewed by IARC is presented in the following sections.

4.1 Conjugated estrogens

IARC concluded that there is limited evidence to evaluate the carcinogenicity of conjugated estrogens in animals (IARC 1979, 1987, 1999; Appendices A, B and C).

Groups of 20 male and female weanling Sprague-Dawley rats were fed diets containing conjugated estrogens (Premarin) at 0, 0.07, or 0.7 mg/kg body weight (b.w.) per day for two years (Gibson et al., 1967). Mammary, pituitary, and thyroid tumors were reported in treated and control animals. These data were considered insufficient to evaluate the carcinogenicity of conjugated estrogens (IARC 1979).

Subcutaneous administration studies were conducted in hamsters with equilin, *d*-equilenin, or deconjugated hormones (estrone, equilin and *d*-equilenin), and premarin. Microscopic renal carcinomas were detected in animals treated with equilin, estrone, equilin and d-equilenin, and premarin but not in those treated with d-equilenin alone (Li *et al.* 1983, 1995).

4.2 Estradiol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of estradiol-17β in experimental animals (IARC 1979, 1987, 1999; Appendices A, B, and C).

Dietary administration of five ppm estradiol to female mice increased the incidences of endometrial preneoplastic lesions and adenocarcinomas, cervical adenocarcinoma, cranial osteosarcoma, adenoacanthoma of the uterus, and mammary adenocarcinoma in female mice (Niwa et al. 1991; Highman *et al.* 1977, 1980). Administration with drinking water doses of 0.5 mg/L estradiol-17β to groups of female C3H/HeJ (MTV^+) mice resulted in a significantly increased incidence of mammary tumors and benign vaginal stromal polyps (Welsch *et al.* 1977, Sheehan *et al.* 1982).

Increased incidence of mammary tumors were observed in mice following subcutaneous implantation with one to five mg estradiol (Rudali 1975, Rudali *et al.* 1978).

In rats, subcutaneous doses of 5 mg estradiol caused increases in the incidence of pituitary tumors females while administration with subcutaneous doses of 27.5 mg induced

increased incidence of both pituitary and mammary gland tumors (Satoh *et al.* 1997, Shull *et al.* 1997). No increase in the incidence tumors were seen in rats given 0.1 mg subcutaneous estradiol-3-benzoate (Shellabarger and Soo, 1973). Subcutaneous implantaion of rats with 5 mg/mL estradiol also did not induce increased incidence of benign vaginal stromal polyps tumors (Sheehan *et al.* 1982).

In studies in which a limited number of animals were used, renal tumors were observed in castrated male and ovariectomized female Syrian hamsters administered 20 or 25 mg subcutaneous doses of estradiol (Kirkman 1959; Li *et al.* 1983; Liehr *et al.* 1986; Li and Li 1987; Goldfarb and Pugh 1990).

4.3 Estriol

IARC concluded that there is *limited evidence* for the carcinogenicity of estriol in animals (IARC 1979, 1987, 1999 Appendices A, B, and C).

Subcutaneous implantation of estriol was not carcinogenic in rats (IARC 1999, Appendix A). In mice, increased incidence of mammary tumors was seen in castrated males and females subcutaneously implanted with estriol (0.64-0.85 mg estrogen) (Rudali 1975). Increased incidence of renal tumors were seen in hamsters of heterogenous origin subcutaneously exposed to 20 mg pellets of estriol (Kirkman 1959).

4.4 Estrone

IARC concluded that there is *sufficient evidence* for the carcinogenicity of estrone in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

In rats, mammary gland tumors were seen following subcutaneous implantation with estrone (Dunning *et al.* 1953, Cutts 1966). Pituitary tumors and adrenal carcinomas were also seen in rats following subcutaneous doses of estrone (Geschickter and Byrnes 1942; Chamorro 1943; Noble *et al.* 1975).

In mice, drinking water doses of 125 or 2,000 μg/L estrone resulted in high incidences of mammary gland tumors (33/68 and 119/169, no control data given) (Boot and Muhlbock 1956). The incidence of mammary tumors was also observed to increase in castrated male mice given 6 μg/day dietary estrone (Rudali *et al.* 1978). Mammary tumors were found in male and female mice given subcutaneous doses of estrone (Bonser 1936; Shimkin and Grady 1940; Bittner 1941).

Intact and castrated male Syrian hamsters given subcutaneous implantations of estrone have been reported to develop significant numbers of renal tumors (Dontenwill 1958; Kirkman 1959; Li *et al.* 1983).

4.5 Synthetic estrogens

4.5.1 Ethinylestradiol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of ethinylestradiol in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

Oral administration of ethinylestradiol produced benign liver tumors in male and female rats and malignant liver tumors in female rats (Committee on Safety of Medicines 1972, Ogawa *et al.* 1995). Female Mead-Johnson rats fed 53 µg/day of ethinylestradiol did not develop any tumors (McKinney *et al.* 1968).

Groups of 120 CF-LP (MTV^+) mice were given ethinylestradiol at 2 to 400 times the human dose. Pituitary tumors were observed in 26 males and 38 females compares to two and eight tumors in control male and female mice, respectively (Committee on Safety of Medicines 1972). A small increase in the incidence of pituitary tumors (both sexes), mammary tumors (both sexes), cervical tumors, and benign gonadal tumors (males) was also reported in BDH-SPF mice (Committee on Safety of Medicines 1972).

Female dogs treated with a combination of ethinylestradiol and norgestrel at 10 to 25 times the human dose had and increased incidence of mammary nodules (Finkel and Berliner 1973).

4.5.2 Mestranol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of mestranol in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

Unspecified doses of mestranol via an unspecified route induced increased incidence of mammary tumors in rats (Committee on Safety of Medicines 1972). Female Sprague-Dawley rats given 6 or 30 µg/kg b.w. of mestranol in the diet developed hepatic nodules and hepatocellular carcinomas (Yager *et al.* 1984).

Dietary mestranol given to castrated male mice at doses from 0.075 to 1 mg/kg b.w. per day developed mammary tumors (Rudali *et al.* 1971). Pituitary tumors were increased in mice of both sexes given 2 to 400 times the human dose in the diet (Committee on Safety of Medicines 1972)., Barrows et al. (1977) reported no increase in hepatocellular tumors in female Swiss Webster or CF-LP mice given 5, 30, 60, or 200 µg/kg b.w. per day.

Female dogs given mestranol at did not show an increased incidence of tumors (Geil and Lamar 1977, Giles *et al.* 1978, Kwapien *et al.* 1980).

A summary of the carcinogenicity studies of steroidal estrogens in experimental animals is presented in Table 4-1.

Table 4-1. Carcinogenic effects of steroidal estrogens in experimental animals[a]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Conjugated estrogen							
Conjugated equine estrogens and equilin	s.c.	hamsters (castrated male), 8–9	20 mg pellet, 9 mo	renal tumors; 6/8	NA	NS	Li *et al.* 1983[b]
Deconjugated hormones (estrone, equilin, *d*-equilenin, Premarin)	s.c.	hamsters (castrated male), 6–8	111 μg/d, 9 mo	estrone, 15 renal tumors equilin + *d*-equilenin, 18 renal tumors Premarin, 16 renal tumors	NA	NS	Li *et al.* 1995[b]
Estradiol							
Estradiol	diet	ICR mice (female), 30–31	5 ppm, 20 wk	NA	endometrial preneoplastic lesions; 48% endometrial adenocarcinoma; 7/31	no endometrial tumors	Niwa *et al.* 1991[b]
Estradiol	diet	ICR mice (female), 41	5 ppm, 16 wk	NA	development of cystic glandular hyperplasia and adenomatous and atypical hyperplasia of the endometrium	NS	Niwa *et al.* 1991[b]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol	diet	C3H/HeJ (MTV^+) mice[d] (female), 200–227	100, 1,000, 5,000 µg/kg, 104 wk	NA	cervical adenosis; 1,000 µg/kg, 8/20 5,000 µg/kg, 3/6 uterine adenocarcinoma; 5,000 µg/kg, 5/207 mammary hyperplastic alveolar nodules; 5,000 µg/kg, 6/17 (wk 95–105) mammary adenocarcinoma; 5,000 µg/kg, 8/17 (wk 95–105)	cervical adenosis; NR uterine adenocarcinoma; 0/227 mammary hyperplastic alveolar nodules; 6/50 (wk 95–105) mammary adenocarcinoma; 19/50 (wk 95–105)	Highman *et al.* 1980[b]
Estradiol	diet	C3H/HeJ (MTV^+) mice[d] (female), 48	100, 1,000, 5,000 µg/kg, 24 mo or 104 wk	NA	mammary adenocarcinoma: 100 µg/kg, 0/35 1,000 µg/kg, 6/36 5,000 µg/kg, 8/48 100 µg/kg, 1 cervical adenocarcinoma, 1 cranial osteosarcoma 5,000 µg/kg, 2 uterine adenocarcinoma, 3 cervical adenocarcinoma 1 adenoacanthoma	mammary adenocarcinoma; 4/47	Highman *et al.* 1977
Estradiol	drinking water	C3H/HeJ (MTV^+) mice (female), 99	0.5 mg/L, 19 mo	NA	mammary tumors; 27/99	mammary tumors; 11/100	Welsch *et al.* 1977

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol dipropionate	s.c. injection	Fischer 344 rats (female), 2–16	5 mg, once every 2 wk for 13 wk	NA	pituitary adenoma; 11/12 (wk 7) carcinoma; 16/16 (wk 13)	0/10 tumors	Satoh *et al.* 1997[b]
Crystalline estradiol	s.c. implant	ACI rats (intact female, ovariectomized female), 21	27.5 mg, 197 d	NA	mammary carcinoma; intact, 21/21 pituitary tumors; similar incidence in intact and ovariectomized	0/3	Shull *et al.* 1997[b]
Estradiol	s.c. implant	Sprague-Dawley rats (ovariectomized female), 19	0.5 mg/L, 16 mo	NA	benign vaginal stromal polyps; 0/17	NS	Sheehan *et al.* 1982[b]
Estradiol-3-benzoate	s.c. injection	Sprague-Dawley rats (female)	0.1 mg	NA	no tumors	NS	Shellabarger and Soo 1973
Estradiol	s.c. implant	(C3H x RIII)F1 (MTV^+) (castrated male) mice	1, 2.5, 5, 10, 100 µg	mammary tumors; 1 µg, 11/31 2.5 µg, 23/27 5 µg, 24/27 10 µg, 27/27 100 µg, 24/24	NA	mammary tumors; 11/33	Rudali *et al.* 1978
Estradiol	s.c. injection	Syrian golden hamsters (castrated male), 5	20 mg, 5.3 mo	renal carcinoma	NA	no tumors	Goldfarb and Pugh 1990[b]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol	s.c. injection	Syrian golden hamsters (castrated male), 6	20 mg, 8.3 mo	renal carcinoma	NA	no tumors	Li *et al.* 1983[b]
Estradiol	s.c. implant	Syrian golden hamsters (castrated male)	25 mg, 6 mo or 9–10 mo	renal-cell carcinoma; 6 mo, 4/5 9 or 10 mo, 6/6	NA	NS	Liehr *et al.* 1986, Li and Li 1987[b]
Estriol							
Estriol	s.c. implant	(C3H x RIII)F1 (MTV^+) mice (castrated male, female)	0.64–0.85 mg	mammary tumors; 25/30	mammary tumors; 18/18	mammary tumors; males, 10/16 females, 28/34	Rudali 1975
Estriol	s.c. implant	hamsters (heterogenous)	20 mg, 318–601 d	renal tumors; 6/11	NA	NS	Kirkman 1959
Estrone							
Estrone	drinking water	C3H mice C3He (MTV^-) mice	125 or 2,000 µg/L	NA	mammary gland tumors; C3H mice, 33/68 (C3He) (MTV^-) mice, 119/169	NS	Boot and Muhlbock 1956[b] (and cited in IARC 1979)
Estrone	diet	(C3H x RIII)F1 (MTV^+) mice (castrated male)	0.66, 0.6, 6 µg/d	mammary tumors; 11/33 (0.66 µg/day), 15/30 (0.6 µg/day), 33/34 (6 µg/day)	NA	mammary tumors; 12/33	Rudali *et al.* 1978
Estrone	s.c. implant	Sprague-Dawley rats (female)	10% estrone, 370 d	NA	no tumors	no tumors	Lemon 1975
Estrone	s.c. implant	hooded rats (female)	NS	NA	adrenal cortical tumors; 20%	adrenal cortical tumors; 5%	Noble 1967

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estrone	s.c. implant	hooded rats (female)	10 mg 90% estrone, 10–53+ wk	NA	adrenal carcinoma, mammary carcinoma, pituitary tumors[e]	NS	Noble *et al.* 1975
Estrone	s.c. implant	Fischer 344 rats	≈10 mg	NA	mammary gland tumors; 12/74	NS	Cutts 1966
Estrone	s.c. implant	Wistar rats	≈10 mg	NA	mammary gland tumors; 12/50	NS	Cutts 1966
Estrone	s.c. implant	Lewis rats	≈10 mg	NA	mammary gland tumors; 17/44	NS	Cutts 1966
Estrone	s.c. implant	Sprague-Dawley rats	≈10 mg	NA	mammary gland tumors; 16/38	NS	Cutts 1966
Estrone	s.c. implant	hooded rats	≈10 mg	NA	mammary gland tumors; 182/212	NS	Cutts 1966
Estrone	s.c. implant	AxC rats (male, female)	8–12 mg	mammary gland tumors; 4/30	mammary gland tumors; 3/32	NS	Dunning *et al.* 1953
Estrone	s.c. implant	Fischer rats (male, female)	8–12 mg	mammary gland tumors; 2/29	mammary gland tumors; 3/29	NS	Dunning *et al.* 1953
Estrone	s.c. implant	August rats (male, female)	8–12 mg	mammary gland tumors; 9/25	mammary gland tumors; 5/12	NS	Dunning *et al.* 1953
Estrone benzoate	Subcutaneous injection	Rats (male, female)	50–100 µg, twice weekly for 20 mo	mammary gland tumors; 1/2 pituitary tumors; 100%	mammary gland tumors; 5/8 pituitary tumors; 100%	NS	Chamorro 1943
Estrone	s.c. injection	rats (castrated male, ovariectomized female)	50–200 µg/d, for total dose of 30-40 mg	mammary gland tumors; castrated males, 6/6 intact males, 2/6	mammary gland tumors; ovariectomized females, 4/5 intact females, 3/8	NS	Geschickter and Byrnes 1942

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estrone	s.c. implant	A strain mice (male, female) C3H (MTV^+) mice (female)	2 mg	mammary tumors	mammary tumors	NS	Bittner 1941
Estrone	s.c. implant	Hybrid (A, C3H, C57, JK) mice	1 to 7 mg	lymphoid tumors; 19/105[f]	lymphoid tumors; 19/105[f]	lymphoid tumors; 21/391[g]	Gardner and Dougherty 1944
Estrone benzoate	s.c. injection	A strain (MTV^+) mice	30–50 µg weekly, 43 wk	mammary tumors; 3/21	NA	NS	Bonser 1936
Estrone benzoate	s.c. injection	C3H (MTV^+) mice (male)	50 µg weekly, 24 wk	mammary tumors; 2/10	NA	NS	Shimkin and Grady 1940
Estrone benzoate	s.c. injection	C3H mice (female)	50 µg weekly, 24 wk	NA	mammary tumors; 100%	mammary tumors; 100%	Shimkin and Grady 1940
Estrone	s.c. implant	Syrian hamster (intact and castrated male)	20 mg, 8.5 mo	renal carcinoma; 8/10	NA	NS	Li *et al.* 1983[b]
Estrone	s.c. implant	Syrian hamster (intact and castrated male)	20 mg	malignant renal tumors; intact, 7/8 castrated, 10/10	NA	malignant renal tumors; intact, 0/6 castrated, 0/60	Kirkman 1959[b]
Estrone	s.c. injection	Syrian golden hamsters (castrated male)	NS	malignant renal tumors; 60% pituitary adenoma; 25%	NA	NS	Dontenwill 1958

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Synthetic estrogens							
Ethinylestradiol	diet	Mead-Johnson rats, 30 (females)	53 µg/kg per day	NA	no increase in any type	NS	McKinney *et al.* 1968
Ethinylestradiol	NS	rats, 73–120	low, med, high (2–400 X human dose)	benign liver-cell tumors; 15%	benign liver-cell tumors; 23%	0 to 8% benign liver-cell tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	rats, 73–120	low, med, high (2–400 X human dose)	NA	malignant liver-cell tumors; 7.5%	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	gavage	Wistar rats, 23-26 (females)	75 or 750 µg	NA	hepatocellular carcinomas; 2 (low dose), 10 (high dose)	no tumors	Ogawa *et al.* 1995[b]
Ethinylestradiol	Diet	CF-LP (MTV^+) mice, 120	low, med, high (2–400 X human dose)	pituitary tumors, 26	pituitary tumors, 38	pituitary tumors; males, 2 females, 8	Committee on Safety of Medicines, 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	pituitary tumors; 4%	pituitary tumors; 10%	pituitary tumors; males, 2% females, 0%	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	benign gonadal tumors; 8 to 10%	NA	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	mammary tumors; 9%	mammary tumors; 32%	mammary tumors; males, 0% females, 3%	Committee on Safety of Medicines 1972

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	NA	uterine or cervical tumors; 4 to 11%	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol + norgestrel	NS	dogs, 12 (females)	10–25 X human dose	NA	mammary nodules; 8 (33.3%)	mammary nodules; 2 (16.7%)	Finkel and Berliner 1973
Mestranol	NS	rats 100 50 controls (females)	unspec.	NA	mammary tumors; 22%	mammary tumors; 5%	Committee on Safety of Medicines 1972
Mestranol	diet	Sprague-Dawley, 15-16 (females)	6 or 30 µg/kg per day	NA	hepatic nodules and carcinomas; 4 (25%)	none	Yager *et al.* 1984[b]
Mestranol	diet	RIII (MTV^+) mice (males), 13–19	0.1 mg/kg per day	mammary tumors; castrated, 11 (84.6%) intact, 8 (42.1%)	NA	NS	Rudali *et al.* 1971
Mestranol	diet	C3H x RIII) F_1 (MTV^+) mice (castrated), 26–41	1 mg/kg per day	mammary tumors; 24 (92.3%)	NA	mammary tumors; 7 (17.1%)	Rudali *et al.* 1971
Mestranol	diet	C3H x RIII) F1 (MTV^+) mice (castrated), 32–61	0.075 mg/kg per day	mammary tumors; 26 (81.3%)	NA	mammary tumors; 10 (16.4%)	Rudali *et al.* 1972
Mestranol	diet	CF-LP mice, 120–240	low, med, high (2–400 X human dose)	pituitary tumors; 12 (10%)	pituitary tumors; 17 (14.2%)	pituitary tumors; males, 4 (1.7%) females, 12 (5%)	Committee on Safety of Medicines 1972
Mestranol	diet	Swiss mice, 47–123	low, med, high (2–400 X human dose)	mammary tumors; ≈ 4%	mammary tumors; ≈ 4%	no tumors	Committee on Safety of Medicines 1972

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Mestranol	NS	Swiss Webster and CF-LP mice (females), unspec.	5, 30, 60, or 200 µg/kg per day	no increase in hepatocellular tumors	no increase in hepatocellular tumors	NS	Barrows *et al.* 1977
Mestranol	NS	dogs (females), 13-20	10–25 X human dose	NA	mammary adenoma; 1	benign mixed mammary tumors; 2	Geil and Lamar 1977, Giles *et al.* 1978
Mestranol	NS	beagle dogs (females), 15	0.02 or 0.05 mg/kg per day	NA	none	NS	Kwapien *et al.* 1980[b]
Mestranol	oral	monkeys (females), 16–20	2, 10, or 50 X human dose	mammary nodules; unspec. no. randomly distributed after 7 yr	mammary nodules; unspec. no. randomly distributed after 7 yr	mammary nodules; unspec. no. randomly distributed after 7 yr	Geil and Lamar 1977
Enovid (1.5% mestranol, 98.5% norethynodrel)	NS	Rhesus monkeys (females), 6	1 mg/day	NA	mammary adenocarcinoma; 1	NS	Kirchstein *et al.* 1972

Source: Cited in IARC 1979 unless otherwise noted
[a]NA = not applicable; NS = not specified; NR = not reported.
[b]Cited in IARC 1999.
[c]Although no control tumor incidence data were reported, a zero incidence has been estimated for the experimental conditions of this study (Liehr *et al.* 1986a).
[d]Strain with a high titer of antibodies to the mouse mammary tumor virus.
[e]The incidence of mammary adenomas was increased in treated males and females up to one year, but was lower than that of controls thereafter.
[f]Overall incidence.
[g]Value in corresponding controls.

4.6 Neonatal exposure to estrogens

4.6.1 Mice

Data from several studies on the effects of neonatal estrogen exposure on mouse vaginal tissue suggest that estrogens affect the fornical and cervical tissues of the genital tract, causing irreversible cornifications, downgrowths, adenosis, and adenocarcinomas (Kimura and Nandi 1967, Forsberg 1972, 1973, 1975, 1979, Takasugi 1976, 1979, Jones and Bern 1977, cited in IARC 1979). Increased mammary tumorigenesis has also been reported as a consequence of neonatal exposure of mice to estrogens (estradiol-17β) (Bern *et al.* 1975, 1976, Mori 1968; Mori *et al.* 1967, 1976, Warner and Warner 1975, Jones and Bern 1977, all cited in IARC 1979).

4.7 Summary

Experimental animal studies in rats, mice, and hamsters have been conducted using estrogens alone or in combination with known carcinogens. Estrogen had a carcinogenic effect regardless of the animal model or route of administration. Most studies resulted in induction of benign and malignant neoplasms as well as preneoplastic lesions in a variety of target organs, including the breast and female reproductive tract.

Dietary estradiol and estradiol administered in drinking water were carcinogenic to mice, inducing increased incidence of mammary tumors in females. Increased incidence of mammary tumors was also evident in male mice administered subcutaneous doses of estradiol. In rats, subcutaneous implantations of estradiol increased the incidence of mammary and pituitary tumors in females. Renal carcinomas were observed in hamsters exposed to estradiol via the subcutaneous route.

Mice given subcutaneous implantations of estriol developed mammary tumors while male hamsters of heterogenous origin, similarly treated, developed renal tumors. Subcutaneous implantation of estriol did not induce any carcinogenic effect in rats.

Increased incidences of mammary tumors were observed in both sexes of mice following oral exposure to estrone and in both sexes of rats following subcutaneous exposure. Increases in the incidence of adrenal, lymphoid, pituitary tumors were also evident in rats following subcutaneous exposure to estrone. Hamsters exposed by subcutaneous administration to estrone developed renal tumors.

The synthetic estrogens have also been found to be carcinogenic in experimental animals. In poorly reported studies where routes of administration and/ or doses were not clearly identified, ethinylestradiol, caused mammary, cervical/uterine, and renal tumors in mice while mestranol caused increased incidence of mammary and pituitary tumors in mice.

5 Genotoxicity

The IARC reviewed the literature through 1999 regarding the genotoxicity of sex hormones, hormonal contraceptives, and post-menopausal hormone therapy (IARC 1987, 1999). The relevant genotoxicity information from the IARC (1999) monograph is summarized in Table 5-1. For a more complete review of these data, see Appendices A and B.

Table 5-1 includes results for the two synthetic estrogens, ethinylestradiol and mestranol, that are widely used in oral contraceptives, as well as for endogenous estrogens and metabolites. The most widely studied compounds are the synthetic hormones and estradiol. Results from studies that combined estrogens with other hormones or chemicals are not included in the table but are available for review in Appendix A. In general, estrogens combined with other chemicals did not show genotoxic effects that were not also seen with individual estrogens. One exception was the induction of reverse mutation in bacterial systems exposed to mestranol combined with 2-acetylaminofluorene, nitrosopiperidine, or a progestogen (IARC 1999).

There was no evidence of genotoxic effects in nonmammalian systems (IARC 1999). The most common findings in mammalian systems included DNA adduct formation in laboratory animals (*in vitro* and *in vivo*), transformation in animal cell lines, and aneuploidy in animal and human cell lines. *In vitro* studies with human cell lines, in addition to aneuploidy, gave some evidence of DNA strand breaks, micronucleus formation, and sister chromatid exchange (Table 5-1). No human *in vivo* data were available.

Sections 5.1 through 5.4 present results of genotoxicity studies that were not reviewed in IARC (1999).

5.1 Prokaryotic systems

5.1.1 Gene mutation in Salmonella typhimurium

Neither ethinylestradiol, cyclotriol, nor cyclodiol induced reverse mutation in the Ames assay, with or without S9 metabolic activation (Hundal *et al.* 1997). A modified host-mediated version of this assay also did not show significant mutagenic effects.

5.2 Plants

No information on the genotoxicity of estrogens in plants was found in the published literature.

5.3 Lower eukaryotic systems

No information on the genotoxicity of estrogens in lower eukaryotes was found in the published literature.

Table 5-1. Genetic toxicology and related effects of steroidal estrogens reviewed in IARC (1999)

	Test system and results[a]																			
	In vitro															*In vivo*				
	Animal cells[b]									Human cells[b]						Animals[b]				
Estrogen type	**A**	**C**	**D**	**G**	**I**	**M**	**S**	**T**		**A**	**C**	**D**	**M**	**S**		**A**	**C**	**D**	**M**	**S**
Synthetic																				
Ethinylestradiol	+[1]	–[1]		–	w			?			–[1]						+	+	–[1]	
Mestranol					w[1]						?			+[1]			?	–[1]	+[1]	+[1]
Endogenous																				
Estradiol	+	–	?	–		+	–	+		+	–		+[1]			?	+[1]	?		?
17α-Estradiol																	–[1]			
4-OH-estradiol			+					+[1]										+		
2-OH-estradiol			?					+[1]										–[1]		
Estradiol-3,4-quinone			+[1]															+[1]		
Estrone			–[1]	–														–		
Estrone-3,4-quinone			w[1]									+[1]								
16α-OH-estrone			+[1]					+[1]												
2-OH-estrone			–[1]																	
4-OH-estrone			+																	
Estriol	+[1]		–[1]					–[1]						w						

Source: Adapted from IARC 1999

[a]Blank cells, not tested or not reported; +, predominantly positive responses; +[1], positive response in a single study; w, weak positive responses; w[1], weak positive response in a single study; ?, both positive and negative responses; –, only negative responses; –[1], negative response in a single study

[b]A, aneuploidy; C, chromosomal aberrations; D, DNA damage; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; S, sister chromatid exchange; T, cell transformation

5.4 Mammalian systems

5.4.1 In vitro *assays*

5.4.1.1 Cytogenetic effects

Estrogen-induced aneuploidy and micronuclei have been reported in various animal and human cell types (Pfeiffer and Metzler 1992, Schnitzler and Metzler 1992, Schuler *et al.* 1996, Metzler *et al.* 1996, Sato and Aizu-Yokota 1996). Steroidal estrogens, with peroxidase-mediated oxidation, interfered with microtubule assembly in a cell-free system (Pfeiffer and Metzler 1992). Interaction with microtubular proteins was proposed as a possible mechanism for estrogen-induced aneuploidy. Schnitzler and Metzler (1992) reported that estradiol, 2-hydroxyestradiol, and 4-hydroxyestradiol induced micronuclei in Syrian hamster embryo fibroblasts and sheep seminal vesicles. Schuler *et al.* (1996) reported that estradiol induced micronuclei in human chorionic villus cells. Sato and Aizu-Yokota (1996) tested several natural estrogens and their catechol derivatives for their ability to disrupt the cellular microtubule network in Chinese hamster V79 cells. The effective concentration required to disrupt microtubules in 50% of the cells (EC_{50}) ranged from 2 mM for 2-methoxyestradiol to > 100 mM for estrone. The EC_{50} for the catechol derivatives of estrone ranged from 30 to 70 mM.

Cultured human lymphocytes were exposed to ethinylestradiol, cyclotriol, or cyclodiol at a concentration of 1, 10, or 100 μg/mL, with and without S9 metabolic activation, for 24, 48, or 72 hours (Hundal *et al.* 1997). All three of these oral contraceptive drugs significantly increased chromosomal aberrations without S9 metabolic activation. Ethinylestradiol was the most potent, inducing both chromosomal and chromatid-type aberrations at all doses and durations except at the lowest concentration for the shortest duration. Six-hour exposure in the presence of S9 significantly increased the frequency of chromosomal aberrations at the two highest concentrations.

5.4.1.2 Sister chromatid exchange

17β-Estradiol at a concentration of 10^{-5} M increased the incidence of sister chromatid exchange (SCE) in epithelial cells from the cervix and vagina of neonatal NMRI mice (Hillbertz-Nilsson and Forsberg 1989). Human peripheral blood lymphocyte cultures were exposed to ethinylestradiol, cyclotriol, or cyclodiol at a concentration of 1, 10, or 100 μg/mL for 24 or 48 hours without metabolic activation (Hundal *et al.* 1997). All three estrogens significantly increased SCEs at all concentrations. In separate experiments, cultures were given 90-minute pulse exposures (with or without metabolic activation) at all three concentrations. Significant increases in SCEs were reported for most exposures.

5.4.1.3 DNA damage or repair

In a review article, Liehr *et al.* (1990) reported that the catechol estrogen metabolites were genotoxic *in vitro*, resulting in formation of quinone and DNA adducts. The comet assay (single-cell gel electrophoresis) was used to detect DNA breaks in human peripheral blood lymphocytes and sperm exposed to estradiol (Anderson *et al.* 1997). Exposure of peripheral blood lymphocytes to estradiol at concentrations ≥ 50 μM for 0.5 hours significantly increased DNA damage. Sperm samples were exposed for one hour; exposure to estradiol at concentrations ≥10 μM significantly increased DNA damage.

5.4.2 In vivo *assays*

5.4.2.1 Aneuploidy and micronucleus formation

The incidences of aneuploidy and micronuclei were increased by factors of 8.0 and 4.3, respectively, in estrogen-induced renal tumors in male Syrian hamsters. Endomitosis, chromatid and chromosome breaks, and telomeric associations also were increased in these tumors (Banerjee *et al.* 1992). Micronuclei were induced in bone marrow cells from Swiss albino mice exposed to ethinylestradiol, cyclotriol, or cyclodiol at 1, 5, or 10 mg/kg body weight (b.w.) via a single intraperitoneal (i.p.) injection (Hundal *et al.* 1997).

5.4.2.2 Sister chromatid exchange

Swiss albino mice were exposed to ethinylestradiol, cyclotriol, or cyclodiol at 1, 5, or 10 mg/kg b.w. via a single i.p. injection (Hundal *et al.* 1997). After 30 hours, the animals were sacrificed, and bone marrow cells were examined for SCEs. Each drug induced a dose-dependent increase in the frequency of SCEs.

5.4.2.3 DNA adduct formation

DiAugustine *et al.* (1992) observed multiple DNA adducts in kidneys from adult male Syrian golden hamsters in both control and estrogen-exposed groups. Chronic subcutaneous exposure to estrogens characterized as strongly carcinogenic (diethylstilbestrol, 17β-estradiol), weakly carcinogenic (ethinylestradiol), or noncarcinogenic (17α-estradiol, β-dienestrol, indanestrol) did not alter the DNA adduct profiles. These results call into question the significance of estrogen-induced DNA adducts in hormonal carcinogenesis.

5.5 Summary

Both synthetic and endogenous steroidal estrogens cause damage to chromosomes and DNA. The most frequently reported effects include formation of DNA adducts, cytogenetic alterations (e.g., chromosome and chromatid breaks, micronucleus formation, SCE), aneuploidy, and cell transformation. Most of these effects have been demonstrated in various *in vitro* assays with cultured animal cells or cell-free systems. Fewer effects have been reported in whole-animal studies or in studies with human cells, and no human *in vivo* data were available.

6 Other Relevant Data

Many tissues, particularly the uterus and mammary glands, contain estrogen receptors and respond to estrogen exposure. 17β-Estradiol (estradiol) is the natural ligand for the estrogen receptor and is used as the standard for determining the estrogenicity of other compounds. Two high-affinity, low-capacity forms of the estrogen receptor (α and β) have been identified. The specific function of the β receptor has not been determined; therefore, most of the data regarding binding affinity, receptor-ligand interactions and transcriptional regulation pertain to the α receptor (IARC 1999). Although there is strong evidence that estrogen carcinogenesis is mediated through the estrogen receptor, there is evidence that estrogenic activity alone is insufficient to explain the carcinogenic effects of estrogens in all tissues. For example, the synthetic estrogen ethinylestradiol binds to the estrogen receptor with affinity equal to that of estradiol, but the former is a much weaker carcinogen. In other cases, the target cells do not contain estrogen receptors (Barrett and Tsutsui 1996).

This section summarizes current views on the probable mechanisms involved in estrogen carcinogenicity. Although the discussion focuses on estrogens, combined estrogen–progestin therapies have become more common in recent years. Combination therapies with progestins are used to lower the risk of endometrial cancer, but they do not reduce breast cancer risk (Colditz 1998, Henderson and Feigelson 2000). There is some evidence that taking estrogens in combination with progestins might increase the risk of breast cancer. A possible explanation is that progestin is a mitogen in mammary ductal epithelial cells but not in the uterus (Liehr 1997, Colditz 1998, Henderson and Feigelson 2000).

6.1 Estrogen metabolism

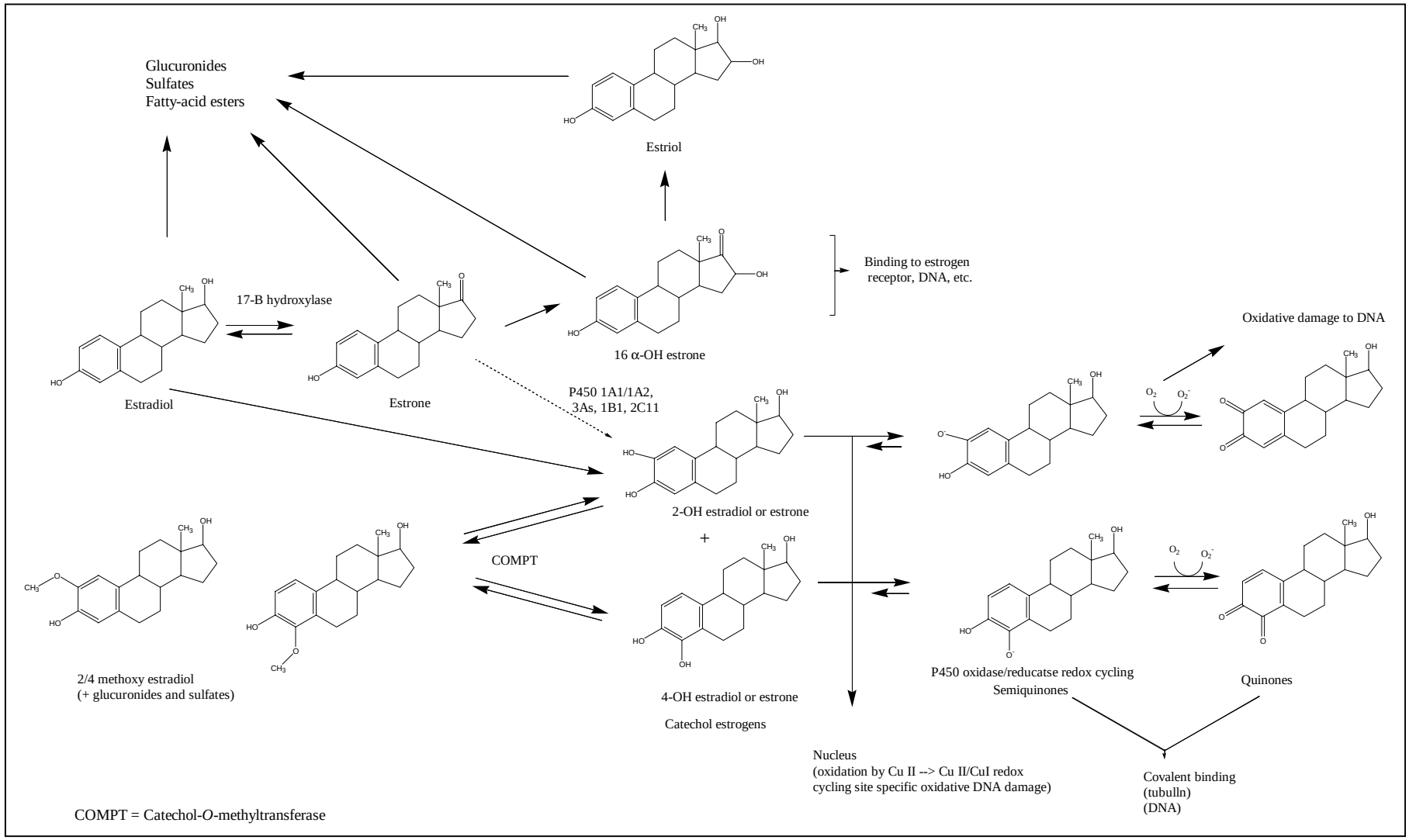
Many different formulations of synthetic and naturally produced estrogens are prescribed for use as oral contraceptives or in postmenopausal hormone replacement therapy. Ethinylestradiol and mestranol are synthetic estrogens commonly used in contraceptives. In the United States, conjugated estrogens are commonly used in postmenopausal estrogen therapy, while in Europe, various preparations of estradiol are preferred. Conjugated estrogens are a mixture of any of at least eight different compounds derived wholly or in part from equine urine or synthetically from estrone and equilin (IARC 1999).

Exogenous estrogens are well absorbed from the gastrointestinal tract and the skin of humans and laboratory animals; therefore, oral, sublingual, dermal, and transdermal preparations are available. The absorption rate, maximum and steady-state concentrations, half-life, and clearance rate depend on the particular estrogen preparation, route of administration, and dose. Estrogens are metabolized in the gastrointestinal tract, liver, and other tissues. It is difficult to make generalizations regarding the pharmacokinetics of estrogens; however, oral administration results in lower circulating levels and faster elimination than dermal or transdermal applications, because of the first-pass effect in the liver (IARC 1999).

In both humans and animals, estradiol, estrone, and estriol undergo similar phase I and phase II reactions. Aromatic hydroxylation reactions catalyzed by cytochrome P-450

enzymes are the primary phase I pathways. Sulfation, methylation, and glutathione conjugation are the major phase II pathways. The ratio of metabolic products depends on the target tissues, species, strain, sex, and experimental conditions (IARC 1999). The primary metabolic pathways for estrogens are illustrated in Figure 6-1 and are discussed in more detail below. The available data indicate that metabolism of conjugated equine estrogens is similar to that of estradiol and estrone; however, conjugated equine estrogens have not been as extensively studied (Bolton *et al.* 1998, IARC 1999).

Figure 6-1. Metabolic pathways for estradiol, estrone, and estriol as adapted from IARC 1999



The major phase I metabolic pathway for endogenous estrogens is aromatic hydroxylation to catechol intermediates. The catechol intermediates have binding affinities for the estrogen receptor similar to the binding affinity of estradiol and undergo cytochrome P-450–mediated redox cycling reactions (Yager and Liehr 1996, Bolton *et al.* 1998, IARC 1999). Phase II reactions include glucuronidation, sulfonation, and *O*-methylation (Figure 6-1). Estrone sulfate is found at the highest concentration in plasma. Sulfate conjugates bind to albumin and circulate in the blood; glucuronides are excreted in urine and bile and may undergo enterohepatic recirculation (IARC 1999).

Estrone and estradiol are biochemically interconvertible and yield the same metabolic products (Figure 6-1). Hydroxylation in the liver by various P-450 isozymes at the 2-position is favored over hydroxylation at the 4-position by a factor of 2 to 10 in all species tested and is greater in women than in men (Bolton *et al.* 1998). The catechol intermediates are further oxidized to semiquinones and quinones. Quinones are highly reactive and can covalently bind to DNA and tubulin (Yager and Liehr 1996, IARC 1999). The catechol intermediates may be detoxified by catechol *O*-methyltransferase (COMT). COMT is present in most tissues and converts catechols into their corresponding methyl ester metabolites. Recent data suggest that 2-methoxyestradiol may inhibit breast cancer (Zhu and Conney 1998). Furthermore, inhibition of COMT potentiates carcinogenicity in the hamster kidney; however, its role in steroid hormone–associated cancers in humans has not been studied (Yager and Liehr 1996).

Evidence links the metabolites of 4-hydroxyestrone (4-OHE) and carcinogenesis. In male Syrian golden hamsters, 4-OHE is carcinogenic, but 2-OHE is not. Furthermore, 4-OHE formation is favored, in all species tested, in tissues that are susceptible to tumor induction by estrogens (e.g., hamster kidney, mouse uterus, and rat pituitary). The liver, where formation of 2-OHE is favored, is more resistant to estrogen carcinogenesis (Bolton *et al.* 1998).

Estrone also may be hydroxylated at the 16α-position to form 16α-hydroxyestrone (Figure 6-1). Although this metabolite's binding affinity for the estrogen receptor is lower than that of the catechol estrogens, it initiates a strong response in growth-promoting genes (Yager and Liehr 1996, Bolton *et al.* 1998). 16α-Hydroxyestrone also alkylates amino acid residues and binds DNA *in vitro* (Yager and Liehr 1996). There are conflicting data regarding the role of 16α-hydroxyestrone in breast cancer in humans (Service 1998).

Conjugated equine estrogens are hydrolyzed to their free forms in the gastrointestinal tract and are absorbed and metabolized in the liver before entering the bloodstream. The dissolution rate affects where the active ingredients are released in the gastrointestinal tract and may ultimately affect the pattern of active and inactive metabolites. The metabolism of equilin and equilenin corresponds to the interrelation between estrone and estradiol (Figure 6-1) (IARC 1999). Although there have been few metabolism studies of equine estrogens, the available data indicate that the relative rates of 2- and 4-hydroxylation differ from those for estrone and estradiol. Studies with baboon, rat, and hamster microsomes show that 2-hydroxylation is the primary metabolic pathway for estrone, but 4-hydroxylation predominates with equilenin (Bolton *et al.* 1998).

6.2 Risk factors and endogenous estrogen

Epidemiological and animal studies have identified estrogen exposure as a risk factor for several cancers. Much of the evidence comes from the observation that cancer risk increases with increased exposure to endogenous estrogens (early menarche or late menopause) or exogenous estrogens (oral contraceptives or hormone replacement) (see Section 3), and a positive relationship between blood levels of estrogens and breast cancer risk (Bolton *et al.* 1998, Colditz 1998).

Obesity is associated with an increased risk of postmenopausal endometrial and breast cancer (Boyd 1996, Colditz 1998). This has been attributed to increased endogenous estrogen production by fat tissue, because fat cells can metabolize androgens to estrogens. Therefore, the relative contribution of estrogen replacement therapy to post-menopausal estrogen concentrations is likely to be greater in thin women than obese women (IARC 1999). Some studies have shown a greater effect of estrogens in obese women, and others have shown a greater effect in thin women (see Section 3). Smoking, for individuals who also are slow acetylators, and alcohol consumption may increase the breast cancer risk from postmenopausal estrogen therapy (Zumoff 1998). An Oxford University study reanalyzed the data from 51 epidemiological studies, which included over 52,000 women with breast cancer and over 100,000 women without breast cancer. This study indicated a positive association between duration of exogenous hormone use (primarily unopposed estrogens) and breast cancer (2.3% increase in risk for each year of use) (Colditz 1998). Other factors, including dosage, type of estrogen, regimen of use, route of administration, ovarian status, and family history, have not shown consistent risk patterns (Brinton and Schairer 1993, IARC 1999).

6.3 Molecular mechanisms

The molecular mechanisms responsible for estrogen carcinogenicity are not well understood. The most widely proposed mechanisms include mitogenesis in cells expressing estrogen receptors, direct genotoxic effects, and indirect effects (Barrett and Tsutsui 1996, Yager and Liehr 1996, Bolton *et al.* 1998). The evidence indicates that estrogen carcinogenesis is complex and involves proliferative effects as well as direct and indirect genotoxic effects. The relative importance of each mechanism is likely a function of the exposed tissue or cell type and its metabolic state (Yager and Liehr 1996).

6.3.1 Cell proliferation and promotion

The endometrium, breast, and liver possess estrogen receptors. Prolonged estrogen exposure induces DNA synthesis and cell proliferation in these tissues and appears to be responsible for tumor formation (Bolton *et al.* 1998). Cell proliferation can facilitate carcinogenesis by increasing the probability that mutations are fixed, thus allowing for clonal expansion of preneoplastic cells. Several lines of evidence support the role of cell proliferation in estrogen carcinogenesis: hormonal influence on the growth of transplanted tumors, estrogen promotion of carcinogen-initiated tumors, and evidence for late-stage effects in human breast cancer (Barrett and Tsutsui 1996). For example, epidemiological studies show an increased risk of breast cancer with current use of estrogen replacement therapy, whereas the risk of breast cancer in women who had stopped taking hormones for

at least five years was no greater than the risk among those who had never taken hormone treatments (Colditz 1998).

Nandi *et al.* (1996) hypothesized that the hormonal environment present during fetal development determines the proportion of mammary epithelial cells that later proliferate as a direct response to hormones. Two types of luminal mammary epithelial cells develop, some with estrogen receptors and some without. Hormones directly stimulate the cells with estrogen receptors to proliferate and to produce growth factors. These growth factors can stimulate proliferation of cells without estrogen receptors. The ratio of replicating cells with and without estrogen receptors at the time of carcinogen exposure determines the eventual frequencies of hormone-dependent and hormone-independent tumors.

6.3.2 Direct genotoxic effects

In addition to the long-recognized mitogenic effects of estrogens, evidence is accumulating that some estrogen metabolites may be directly responsible for the initial genetic damage leading to tumors (Service 1998). 16α-Hydroxyestrone, 4-hydroxyestradiol, and 4-hydroxyestrone are the primary estrogen metabolites that have been associated with direct genotoxic effects and carcinogenicity (Yager and Liehr 1996, Bolton *et al.* 1998, Service 1998, IARC 1999). The evidence for a role of these metabolites in carcinogenicity is reviewed below.

Cultured breast cells exposed to 16α-hydroxyestrone have shown increased DNA repair rates, and this metabolite has been detected in and around breast tumors (Service 1998). In mouse mammary epithelial cells, 16α-hydroxyestrone caused a small but significant increase in unscheduled DNA synthesis, hyperproliferation, and increased colony growth in soft agar, effects not observed with estradiol and estriol (Yager and Liehr 1996). In addition, covalent binding of 16α-hydroxyestrone to DNA *in vitro* has been demonstrated (Yager and Liehr 1996, Service 1998). Increased levels of 16α-hydroxyestrone may increase the risk of breast cancer by increasing both cell proliferation and direct DNA damage. However, the role of 16α-hydroxyestrone in breast cancer is not certain. Some studies have reported that estrogen metabolism favoring formation of 16α-hydroxyestrone over 2-OHE increases breast cancer risk, but other studies have not found this effect (Fishman *et al.* 1995, Yager and Liehr 1996, Bolton *et al.* 1998, Meilahn *et al*. 1998, Zumoff 1998, Ursin *et al.* 1999).

Liehr (1997) described mechanistic similarities between human breast cancer and estrogen-induced kidney cancer in hamsters, and identified metabolism to the 4-hydroxylated catechols as the primary pathway leading to tumor development. The 4-hydroxylated catechols may undergo subsequent redox cycling between semiquinone and quinone forms. The quinones may undergo nonenzymatic isomerization to quinone methides. The quinone and quinone methide intermediates are highly reactive and may form covalent DNA adducts; thus, these metabolites are candidates for the ultimate estrogen carcinogens (Bolton *et al.* 1998). Furthermore, redox cycling generates superoxide radicals that are capable of direct and indirect damage to DNA (see Section 6.3.3). Supporting evidence includes higher levels of urinary catechol estrogens in women at risk of breast cancer than in controls, predominance of 4-hydroxylation over 2-hydroxylation in breast cancer cells,

and induction of kidney and liver tumors in laboratory animals by 4-hydroxylated catechols (Liehr 1997, Service 1998).

6.3.3 Indirect effects

6.3.3.1 Reactive oxygen species

Excessive production of reactive oxygen species has been reported in breast cancer tissue, and free-radical toxicity (DNA single-strand breaks, lipid peroxidation, chromosomal abnormalities) has been reported in hamsters treated with estradiol (Bolton *et al.* 1998). Reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals, may be produced through redox cycling between the *o*-quinones and their semiquinone radicals (Figure 6-1). These reactive oxygen species can cause oxidative cleavage of the phosphate–sugar backbone and oxidation of the purine and pyrimidine residues of DNA. Incubation of 4-hydroxylated catechols with microsomes, NADPH, and DNA resulted in 8-hydroxylation of guanine bases (Yager and Liehr 1996). 8-Hydroxydeoxyguanosine is a biomarker for oxidative damage and is considered an important factor in carcinogenesis (Yager and Liehr 1996, Bolton *et al.* 1998). Hamsters given both estradiol and antioxidants had significantly fewer tumors than those receiving estradiol alone (Bolton *et al.* 1998).

Another possible mechanism for generation of reactive oxygen species is copper-mediated metabolism (Figure 6-1). Copper is present throughout the body and is particularly associated with guanine-rich DNA sequences. The divalent copper ion can oxidize the catechol estrogens, resulting in oxidative damage to DNA (Yager and Liehr 1996).

6.3.3.2 Protein binding

In addition to directly binding to DNA, reactive estrogen metabolites may form covalent bonds with proteins. Covalent binding of quinones to microtubular proteins is a possible mechanism for aneuploidy and cell transformation reported in animal *in vitro* studies. Covalent binding of estrogen quinone metabolites to tubulin has been demonstrated *in vitro* (Yager and Liehr 1996).

6.3.3.3 Protooncogene regulation and genetic susceptibility

Protooncogenes are involved in normal cell growth and development; however, overexpression can lead to cell transformation. Hyder *et al.* (1992) identified several uterine protooncogenes regulated by estradiol, including c-*fos*, c-*jun*, c-*myc*, N-*myc*, *ras*, and *erb* B. Chronic administration of estrogens to male Syrian hamsters resulted in 100% incidence of kidney tumors. The mRNA levels of c-*fos*, c-*jun*, and c-*myc* in the tumors were 14, 6, and 4 times higher than levels in the controls. However, these researchers also noted that protooncogene overexpression in target tissues could be due to estradiol; other physiological, pharmacological, or toxicological agents; or a combination of these agents and estradiol. Therefore, breast and endometrial cancer could result from *fos* overexpression even if the endocrine profile were normal. Estrogen-regulated events also occur throughout the cell cycle. Estrogens rapidly stimulate expression of protooncogenes associated with the G_0 to G_1 transition, but later stimulate expression of other genes that are associated with progression through G_1 to S phase. In hormonal carcinogenesis, the tumor phenotype would depend upon the affected estrogen-regulated event (Hyder *et al.* 1992).

Boyd (1996) reported some evidence for K-*ras* involvement in estrogen-related endometrial carcinomas. This *ras* mutation was observed in 10% to 30% of human endometrial carcinomas and appeared to be an early event. However, the data also indicated that hyperplastic lesions with the *ras* mutation were no more likely to progress to carcinoma than those without it.

The expression of c-*myc*, c-*fos*, c-*jun*, and c-*myb* in the uterus and mammary gland is altered rapidly in response to estrogens. Li *et al.* (1999) demonstrated that the expression of these genes increased in the Syrian hamster kidney and renal tumors after five to six months of continuous estrogen exposure. The c-*myc* gene in particular appears to play a critical role in abnormal cell proliferation, cell immortalization, and neoplastic development. Increased expression of this gene may be due in part to a gain in chromosome number. Chromosome 6qb, which contains the c-*myc* gene, had a high frequency of trisomies and tetrasomies after five months of estrogen exposure.

Serum estradiol level variations in part may be explained by genetic differences. For example, North American women have higher blood levels of estradiol and a higher incidence of breast cancer than Asian women. The specific genes involved in hormone-related cancers are unknown; however, candidate genes include those involved in the endocrine pathways, DNA repair, or tumor suppression, as well as oncogenes (Henderson and Feigelson 2000). Polygenic models of endometrial and breast cancer, developed to help define a high-risk profile for hormone-related cancers, identified several genes involved in estrogen biosynthesis, intracellular binding, and transport. These included genes for 17β-hydroxysteroid dehydrogenase 1 (*HSD17B1*), cytochrome P-459c17α (*CYP17*), aromatase (*CYP19*), and the estrogen receptor alpha (*ER*). Although environmental factors do influence the lifetime hormone burden of an individual, endogenous hormone levels also have a genetic basis that can be an important risk factor for hormone-dependent tumors.

6.4 Summary

The presence of estrogen receptors within certain tissues and tumors and the association between duration of exposure to endogenous or exogenous estrogens and tumor probability indicate that estrogens influence tumor growth in these tissues. Prolonged estrogen exposure induces cell proliferation in estrogen-dependent target cells, affects cellular differentiation, and alters gene expression. However, there is increasing evidence for both direct and indirect genotoxic effects of estrogens. Endogenous and exogenous estrogens are metabolized to electrophilic metabolites capable of binding intracellular proteins and DNA. Furthermore, redox cycling pathways can generate reactive oxygen species, which may cause oxidative damage to DNA. Therefore, in some cases, estrogens may initiate as well as promote carcinogenesis.

7 References

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Appendix A: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Hormonal Contraception and Post-menopausal Hormonal Therapy. V 72. 1999.

Appendix B: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs. Suppl. 7. 1987.

Appendix C: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Sex Hormones (II). Vol 21. 1979.

Appendix D: Report on Carcinogens (RoC), 9th Edition, Profile for Estrogens.